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"STRUCTURAL STUDIES ON THE ANAPLASTIC LYMPHOMA KINASE"

Dr. Carla Donadoni

Coordinator: Prof. Enrico Maria Pogliani

Tutor: Prof Carlo Gambacorti Passerini

Dr Carmen Julia Tartari

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ABSTRACT

The ALK tyrosine kinase is expressed, as NMP/ALK fusion protein, in approximately 60% of Anaplastic Large Cell Lymphoma (ALCL) cases and in other cancers such as Non Small Cell Lung Cancer (NSCLC) and neuroblastoma. ALK kinase domain constitutive activation is responsible for the malignant transformation through stimulation of downstream survival and proliferation signalling pathways. A number of ALK inhibitors are under investigation, with crizotinib being recently approved in NSCLC. In particular, acquired resistance to kinase inhibitors is a serious problem in long-term cancer treatment including ALK+ cancers. Structural characterization of the ALK kinase domain and information about drug-protein interactions could be very useful in the rational design of new specific drugs for treatment of the ALK+ ALCL and other ALK+ tumours.

This work has been focused on the production and purification of different forms of the ALK kinase domain to obtain good candidates for ALK structural studies by X-ray crystallography and STD-NMR. r-ALK proteins were expressed in Sf9 (*Spodoptera frugiperda*) insect cells using two different Baculovirus expression systems (Bac-to Bac and BacPAK expression systems). After optimization of the purification procedures, r-ALK proteins (WT and mutant forms) have been characterized obtaining three forms suitable for structural studies. In particular, P e NON-P forms of two purified r-proteins were used to screen about 20000 conditions for crystallization. STD-NMR results of the ALK 6 demonstrated the interaction between the ALK kinase domain and a new kinase inhibitor (R458). In addition, the r-ALK proteins has been used for screening of potential ALK inhibitors (R500B and R458), whose activity has been validated in ELISA kinase assay.

In conclusion, the results of this work could pave the way to the development of new specific drugs for specific treatment of ALK+ diseases.

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INTRODUCTION

PROTEIN KINASES

Protein kinases (PK) are enzymes able to catalyze the transfer of the γ-phosphate group from ATP to the hydroxyl group of serine, threonine and/or tyrosine residues in protein substrates (Hubbard and Miller 2007).

Protein phosphorylation, resulting from PK activity, has an important role in regulating protein function leading to control of signal transduction, metabolism, transcription, cell cycle progression, cytosckeletal rearrangement and cell movement, apoptosis and differentiation (Manning, Whyte et al. 2002).

Human genome sequence analysis has identified about 518 human protein kinases, about 17% of all the human gene, that have been classified into a hierarchy of groups, families and subfamilies. Classification was primarily based on sequence similarity inside and outside the catalytic domain and was also based on comparison of protein biological functions. Actually, protein kinases can be classified as serine/threonine (Ser/Thr) kinases, tyrosine (Tyr) kinases and dual Ser/Thr and Tyr kinases depending on their catalytic action. They are formed by a catalytic domain, approximately 250-300 amino acid, conserved in sequence and structure but with a different regulation. In addition, 83 structural domains have been identified in 258 of the 518 kinases. These domains are able to regulate biochemical activity, subcellular localization and they are able to link the kinase to other signalling molecules (Manning, Whyte et al. 2002).

The three dimensional structure of cyclin adenosine-3’,5’-monophospate (cAMP) dependent protein kinase (PKA), a Ser/Thr protein kinase, was the first to be solved and studied. This represents the basic structure for protein kinases domain, in fact multiple sequence alignments indicate that the catalytic or kinase domain of all PK are extremely well conserved and have similar structure (al-Obeidi, Wu et al. 1998; Manning, Whyte et al. 2002).

The kinase domain has 3 distinct roles: (1) binding and orientation of the ATP (or GTP) phosphate donor as a complex with divalent cation (Mg^{2+} or Mn^{2+}); (2) binding and orientation of the substrate (protein or peptide); (3) transfer of the γ-phosphate from ATP (or GTP) to the acceptor residue of the substrate. The kinase domain is divided into 12 subdomains, defined as regions never interrupted by large amino acid insertions, that form a two-lobed structure linked together by the subdomain V. The smaller N-terminal lobe, including subdomains from I to IV and composed of 5 antiparallel β-sheet structure and one α-helix (called αC-helix), is

primarily involved in anchoring and orienting the nucleotide. The larger C-terminal lobe, with subdomains from VIa to XI and characterized by eight α-helices and four β-strands, is important for binding the peptide substrate and initiating phosphotransfer. In the subdomain VII is the highly conserved DFG motif, located at the beginning of the activating loop (A-loop), which is required for catalytic activity and has a direct role in the phosphor-transfer reaction (Hanks and Hunter 1995) (**Fig 1**). PK activation occurs through phosphorylation or autophosphorylation of residues in subdomain VIII. The phosphorylated-active conformation of the protein permits proper orientation of the substrate peptide, while the unphosphorylation form corresponds to the inactive state (Hanks and Hunter 1995). Asp-166, located in the C-terminal lobe in a loop called catalytic loop, is highly conserved in all kinases (al-Obeidi, Wu et al. 1998).

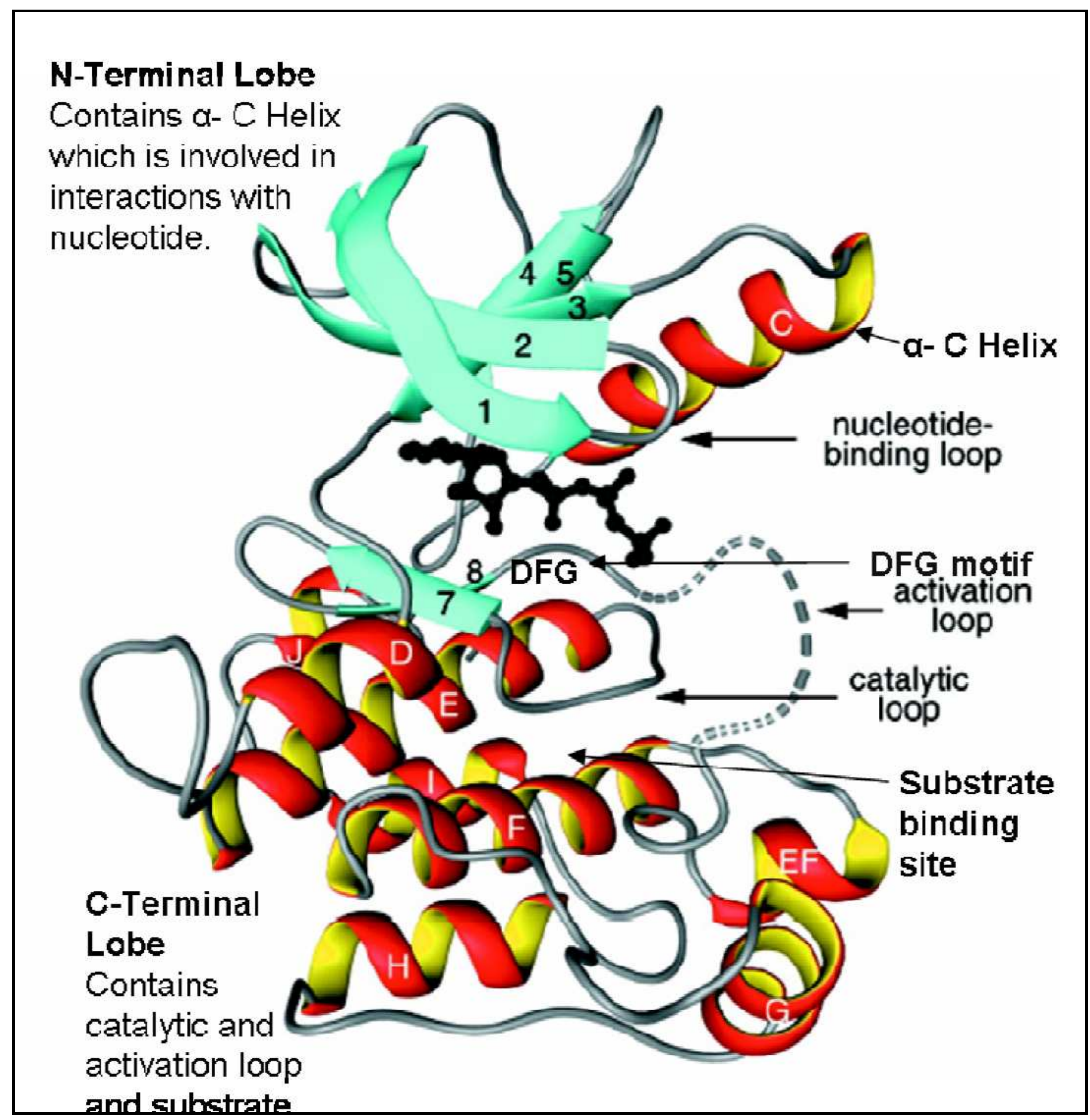

Fig.1_ Structural features of a kinase domain.

Ribbon diagram of the Insulin Receptor kinase domain structure. Strands (numbered) are shown in cyan and helices (lettered) in red and yellow. The nucleotide analog AMP-PCP is shown in ball and stick representation (black). The dashed gray line indicates the portion of the A-loop that is disordered (residues 1155-1171). Figure adapted from (Till, Ablooglu et al. 2001).

PROTEIN TYROSINE KINASES

Protein tyrosine kinases (PTK) catalyze the transfer of the γ phosphate of ATP to the hydroxyl group of tyrosine residues located on protein substrate. There are 58 receptor tyrosine kinases (RTK) divided in 20 subfamilies and 32 non-receptor tyrosine kinase (NRTK) divided in 10 subfamilies (Hubbard and Till 2000; Madhusudan and Ganesan 2004).

RECEPTOR TYROSINE KINASES

RTKs are transmembrane glycoproteins activated by the binding of their specific ligand; their activation leads to the transduction of the extracellular signal to the cytoplasm by autophosphorylation of tyrosine residues on the kinase protein itself or by phosphorylation of downstream signalling proteins. These proteins regulate cell proliferation, differentiation, migration and metabolism. This family includes a lot of growth factor receptors such as the insulin receptor (IR), the epidermal growth factor receptor (EGFR) and the fibroblast growth factor receptor (FGFR) (Hubbard and Till 2000).

In particular, RTKs contain: (1) an amino-terminal extracellular region that binds the ligand, (2) an hydrophobic transmembrane portion and (3) a cytoplasmic domain with tyrosine kinase catalytic activity and regulatory sequences at the C-terminal. The binding of the ligand leads to receptor dimerization/oligomerization and consequent autophosphorylation of tyrosine residues located in the A-loop into the kinase domain, in the juxtamembrane and in the C-terminal region. This autophosphorylation generates docking sites for signal-transducing proteins, as proteins containing SH2 (Src homology2) and PTB (phosphotyrosine binding) domains that recognize phosphotyrosine in specific sequence contexts. Receptor autophosphorylation can occur in *cis*, regarding residues within the receptor itself, or in *trans*, between different receptors. In the first case, the ligand induces a conformational change of the protein that facilitates the autophosphorylation. All the RTKs contain one, two or three tyrosines in the kinase A-loop that are important for the kinase activation (Hubbard and Till 2000) (**Fig 2**).

Crystal structure of different tyrosine kinases is the main source of knowledge about structural organization and mechanism of activation of kinases.

In particular, crystal structures of the unphosphorylated (inactive) and phosphorylated (activated) kinase domain of the IR revealed the main characteristic of proteins belonging to the RTK family (Hubbard, Wei et al. 1994; Hubbard 1997; McKern, Lawrence et al. 2006). The A-loop of the IR kinase has three tyrosine residues (Tyr-1158, Tyr 1162 and Tyr 1163) corresponding to the 'Y-x-x-x-Y-Y' motif. The inactive form of the IR corresponds to the closed conformation of the A-loop. In this case Tyr-1162 forms hydrogen bonds with conserved catalytic aspartic acid and arginine residues creating an intermolecular interaction. The IR uses an autoinhibitory mechanism to maintain the inactive state: the position of the unphosphorylated A-loop prevents protein substrate and ATP to the active site (McKern, Lawrence et al.). The ligand-binding causes trans-autophosphorylation of the IR kinase and conformational changes that stabilize the A-loop in the open-active conformation through hydrogen bonds between pTyr-1163 and the conserved arginine at the beginning of the A-loop and a backbone nitrogen in the latter half of the loop. The stabilization of the A-loop conformation permits to ATP and substrate to reach their binding sites, moreover this conformation facilitates the catalysis by proper orientation of the residues involved in Mg-ATP binding and catalysis (McKern, Lawrence et al. 2006).

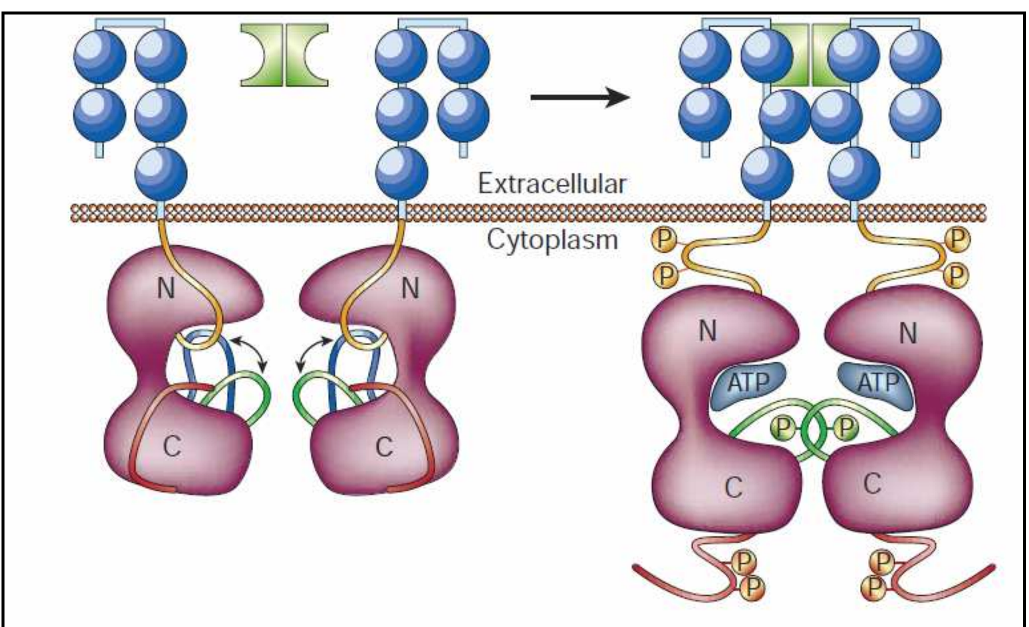

Figure 2_ Structure and activation of Receptor Tyrosine Kinase.

RTK kinase activity is tightly repressed in the unstimulated state. Ligand binding at the extracellular domain induces receptor dimerization and tyrosine autophosphorylation in the kinase domain (in violet) resulting in relief of the inhibitory constraints exerted by the A-loop, and the juxtamembrane and C-terminal regions. N: N-lobe, C: C-lobe. From (Blume-Jensen and Hunter 2001).

NON-RECEPTOR TYROSINE KINASES

NRTKs are integral components of the signalling cascades trigged by RTKs and by other cell surface receptors and receptors of the immune system. The Src family is the largest subfamily of NRTK, it includes nine members involved in different signalling pathways including mitogenesis, T- and B-cell activation and cytoskeleton restructuring (Hubbard and Till 2000). In comparison to RTK, NRTKs do not have an extracellular ligand-binding domain and a transmembrane domain but some of them are anchored to the cell membrane through N-terminal modifications (myristoylation, palmitoylation). Their structure include domains that mediate protein-protein, protein-lipid and protein-DNA interactions. Examples of common protein-protein interaction domains are the Src homology 2 (SH2) that binds phosphotyrosine residues in a sequence specific manner, and the Src homology 3 (SH3) that recognizes proline-containing sequences (Sicheri and Kuriyan 1997; Xu, Harrison et al. 1997).

Activation of NRTKs occurs by phosphorylation or trans-autophosphorylation of tyrosines in the A-loop, while phosphorylation of tyrosine outside the A-loop can lead to protein inhibition (Hubbard and Till 2000).

The c-Src protein, a NRTKs belonging to the Src family, can be considered as a model for all the NRTKs. C-Src has two important regulatory tyrosine phosphorylation sites: Tyr-527 and Tyr-416. Tyr-527 is in the C-terminal tail, its phosphorylation by the NRTK Csk leads to negative regulation of the kinase activity. The interaction between pTyr-527 and the SH2 domain stabilizes the contact of SH3 and a short polyproline type II helix, causing a misalignment of amino acid important for the kinase reaction and protein inactivation. The binding of the ligand disrupts these intramolecular restraints causing kinase protein activation. The second tyrosine regulatory site is Tyr-416, in the A-loop; this is an important autophosphorylation site.

In particular, Abl, a NRTK containing the same c-Src domains in the same order, has a different regulation. The SH3 domain is able to repress the kinase activity, but Abl does not show a C-terminal regulatory phosphorylation site (Superti-Furga 1995) (**Fig 3**).

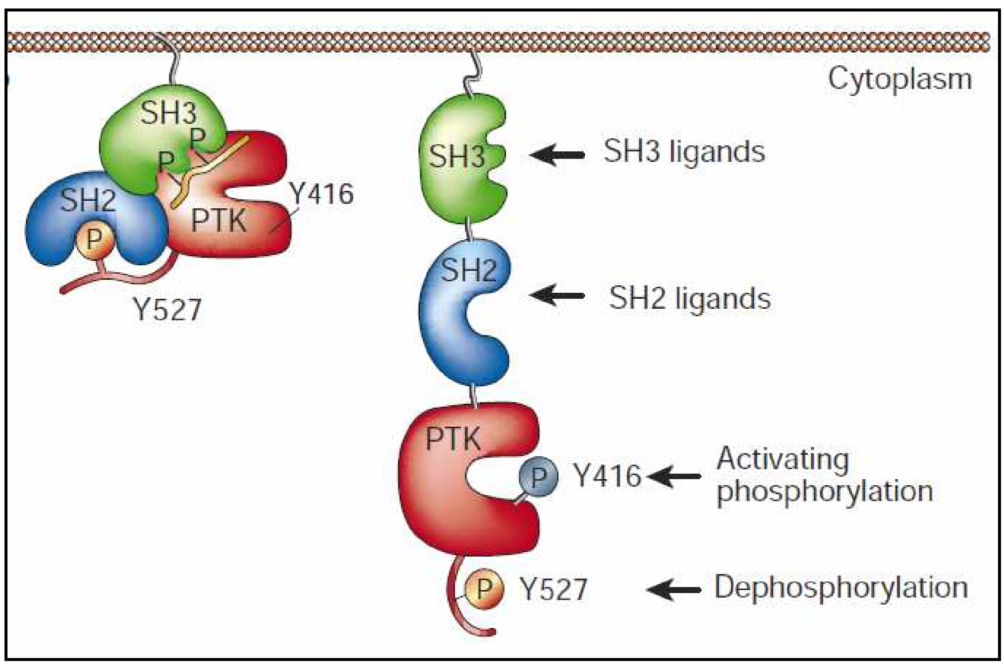

Fig 3_ Structure and activation of Non Receptor Tyrosine Kinase (c-Src)*.*
NRTK activity is tightly repressed in the unstimulated state. The binding of ligands to the SH2 or SH3 domain and/or dephosphorylation of phospho-Tyr 527 by PTPs leads to NRTK activation. From (Blume-Jensen and Hunter 2001).

PROTEIN TYROSINE KINASES AND CANCER

A lot of diseases including cancer, diabetes and inflammation have been found to be correlated to protein tyrosine kinase-mediated cell signalling pathways alteration. This is due to the critical role of protein tyrosine kinases in regulation of fundamental cellular processes. Oncogenic deregulation is the result of perturbation of the auto-inhibitory mechanisms that ensures the normal repression of the catalytic domain activity (Blume-Jensen and Hunter 2001).

Protein tyrosine kinases can acquire transforming ability by several mechanisms:

- Gene amplification or over-expression of PTKs, this happens to the epidermal growth factor receptor (EGFR) and HER-2 in sever cancers and to ALK in the neuroblastoma. The over-expression can increase the probability of monomer dimerization in absence of ligand.
- Genomic rearrangements such as translocations or inversions which can lead to the production of constitutively active fusion proteins as BCR/ABL fusion protein discovered in chronic myeloid leukaemia or NPM/ALK in anaplastic large cell lymphoma.
- Gain of function mutations or deletion within the kinase domain or the extracellular domain which produces constitutively active tyrosine kinase, an example is EGFRvIII mutant with a deletion in the extracellular domain in solid tumours.
- Overexpression of ligands which leads to persistent tyrosine kinase stimulation, as TGF-α overexpression in glioblastoma (Madhusudan and Ganesan 2004).

Considering the prominent role of PTK in different types of cancer, functional study of their kinase domain acquires importance to understand protein role in the diseases and, more important, to give an efficient and definitive cure. Investigating the structure of a PTK responsible for a pathology is a key point in the fight against cancer. In particular a structural study focused on kinase-drug binding can help to improve our knowledge about inhibitors-kinase interaction and to find more efficient and specific drugs.

ANAPLASTIC LYMPHOMA KINASE

The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase, member of the insulin receptor subfamily of RTKs firstly identified in 1994 in the anaplastic large cell lymphoma (ALCL), a non-Hodgkin lymphoma subtype. The t(2;5) chromosomal translocation involving the nucleophosmin gene (npm) creates the NPM/ALK fusion protein (Morris, Kirstein et al. 1994; Shiota, Fujimoto et al. 1994).

ALK FUNCTION

ALK is normally expressed only in the nervous system (thalamus, hypothalamus, mid-brain, olfactory bulb, selected cranial, dorsal root and ganglial) during embryonic development, while in adult mammals the expression is limited to rare neural cells, scattered pericytes and endothelial cells. This indicate a role of ALK in neural development and differentiation (Li and Morris 2008). However, ALK function is not required for the viability of knockout mice (Webb, Slavish et al. 2009), in fact these mice show no apparent developmental, anatomical or locomotor deficits (Bilsland, Wheeldon et al. 2008). They show an hippocampal neurogenesis correlated with the regulation of mood hypothesized to be required for antidepressant efficacy and for several aspects of learning and memory. This evidence suggests an antagonistic role of ALK for the therapeutic treatment of cognitive and mood disorders (Webb, Slavish et al. 2009).

Interestingly, the distribution of ALK mRNA and protein partially overlaps with that reported for members of the Trk receptor, a neurotrophins receptor, suggesting that ALK could serve as receptor for neurotrophic factors (Barbacid 1995). Two proteins have been reported as possible ALK ligands in mouse and man: pleiotrophin (PTN) and midkine (MK), both conserved through evolution (Stoica, Kuo et al. 2001; Stoica, Kuo et al. 2002). Jelly belly (Jeb) instead is the ALK ligand in *Drosophila* (Englund, Loren et al. 2003; Lee, Norris et al. 2003).

PTN is a 18-kDa heparin binding growth factor inducing neurite outgrowth and a mitogenic activity in a wide range of cell lineages. It has a similar distribution and expression in the nervous system compared to ALK (Pulford, Morris et al. 2004). Demonstration of the ALK receptor role of PTN is given by a ligand-receptor binding assays on both cell free extracts and whole cells, moreover PTN is able to increase

ALK substrates phosphorylation in ALK-transfected cells (Stoica, Kuo et al. 2001). Hence, PTN has an important role in development and maintenance of normal neural function. The relationship between ALK and PTN has revealed roles of ALK in cell death regulation as an anti-apoptotic protein via the MAPK pathway (activation of PI3-kinase and PKB/AKT) (Bowden, Stoica et al. 2002). In addition, its role in angiogenesis has been observed (Choudhuri, Zhang et al. 1997). Perez-Pinera *et al.* reported a new mechanism of ALK activation by PTN: the PTN/ PTPRZ1 signalling pathway indirectly phosphorylates ALK in PTN-stimulated cells, so no interaction between ALK and PTN intercourse (Perez-Pinera, Zhang et al. 2007). PTN has other two known receptors: the receptor protein tyrosine phosphatase (RPTP) beta/zeta (Meng, Rodriguez-Pena et al. 2000) and the heparin sulphate proteoglycan N-syndecan (syndecan 3) (Maeda, Nishiwaki et al. 1996).

The second possible human ALK ligand, MK, is a retinoic acid-inducible, developmentally regulated, heparin-binding neurotrophic factor. It shows 45% identity to PTN. MK is involved in the regulation of neurite connections, regulation of neurons migration and angiogenesis as well as PTN. Moreover, as well as PTN, MK expression in the nervous system decreases after birth (Webb, Slavish et al. 2009). MK was first identified as a potential ALK ligand by Stoica *et al*, who demonstrated direct binding of MK to ALK. In addition, this group observed that addition of PTN can compete with MK for ALK binding. In cells, monoclonal antibodies against the extracellular domain of ALK inhibited MK-binding to ALK as well as colony formation of SW-13/MK cells. Furthermore, in different ALK-positive cell lines, MK could induce signal transduction pathways involving PI-3k and MAPK, known downstream targets of ALK (Stoica, Kuo et al. 2002). Also MK, as well as PTN, has other receptors apart from ALK: neuroglycan C (Ichihara-Tanaka, Oohira et al. 2006), PTPRZ1 (Maeda, Ichihara-Tanaka et al. 1999), the low-density lipoprotein receptor-related protein (Muramatsu, Zou et al. 2000) and $\alpha_4\beta_1$- and $\alpha\beta_1$-integrins (Muramatsu, Zou et al. 2004).

ALK STRUCTURE

The genes encoding the full-length ALK protein in mouse and man, located at chromosomal band 2p23 in human and at chromosome 17 in mouse, were cloned in 1997 (Iwahara, Fujimoto et al. 1997; Morris, Naeve et al. 1997).

ALK has an extracellular ligand-binding domain, a transmembrane domain and a cytoplasmic catalytic domain. Because of the high homology between the extracellular region of ALK and the Leukocyte Tyrosine Kinase (LTK), a RTK belonging to the IR superfamily, ALK has been placed in the insulin receptor family (Shiota, Fujimoto et al. 1994; Iwahara, Fujimoto et al. 1997; Morris, Naeve et al. 1997). The ALK size, after post-translational modification such as N-glycosylation, is approximately 200 kDa (Morris, Naeve et al. 1997). ALK is highly conserved across species, in fact between hALK and mALK the homology is 85% while the identity is 34% between hALK and DALK (Pulford, Morris et al. 2004).

Human ALK is a single-chain transmembrane protein of 1620 amino acid (aa) (**Fig 4**) (Iwahara, Fujimoto et al. 1997). The extracellular region, composed of 1030 amino acid, contains a signal peptide sequence at the N-terminal, the binding site for the ligand, a LDL-A domain with unknown function and a MAM domain with a potential role in cell-cell interaction. The intracellular region contains a binding site (aa 1093-1096) for phosphotyrosine-dependent interaction with the IR substrate 1, a domain common in member of the IR superfamily.

The kinase domain (KD) consists of 254 aa (Pulford, Morris et al. 2004). Inside the kinase domain is the catalytic loop including residues 1247-1254 and the A-loop, residues 1270-1299, which begins with the DFG-motif and ends with residues PPE (Lee, Jia et al.). A three-tyrosine-containing motif (Y1278, Y1282, Y1283), also called YxxxYY motif, within the A-loop is in common with the IR. These tyrosines represent the major autophosphorylation site in ALK, activating the kinase via trans-autophosphorylation after dimerization ligand-dependent. In particular the phosphorylation of the first tyrosine residue (Y1278) is predominant in the autoactivation of the ALK kinase domain. Y1283 is also important for the NPM/ALK-SHP1 interaction where SHP1 is a cytoplasmic tyrosine phosphatase with tumour suppressor functions (Hegazy, Wang et al. 2010). The phosphorylation is able to regulate the A-loop conformation: in the unphosphorylated state ATP can not reach the ATP-binding pocket and so the kinase is inactive; while in the phosphorylated state the A-loop moves away from the ATP-binding pocket and the ATP can enter activating the protein (Tartari, Gunby et al. 2008). The gatekeeper residue is a small

hydrophobic pocket at the back of the ATP-binding site, it controls the sensitivity of kinases to small molecules inhibitors. Leucine residues (L1196 in ALK FL) in the gatekeeper sterically impedes the entrance of inhibitors into the ATP-binding site of the activated KD (Gunby, Ahmed et al. 2006). Wang P et al have identified 8 tyrosine residues outside the A-loop by tandem affinity purification in combination with a newly developed, highly sensitive liquid chromatography-mass spectrometry. Despite these phosphorylated tyrosine residues serve as "docking sites" for NPM/ALK downstream effectors, their respectively mutations do not result in a decrease of oncogenity (Wang, Wu et al. 2010).

The 244 amino acids at the C-terminal include a phosphotyrosine-dependent binding site (residues 1504-1507) for the substrate SH2 domain (Degoutin, Vigny et al. 2007), and an interaction site (residues 1603-1606) for the phosphotyrosine-dependent binding of PLC-γ (Bai, Dieter et al. 1998). Phosphorylation of sites outside the A-loop within the cytoplasmic domain serve as docking sites for downstream SH2 and PTB domain-containing effector and adapter proteins involved in the signal transduction cascade.

Recently, Bossi et al (Bossi, Saccardo et al. 2010) and Lee et al (Lee, Jia et al. 2010) crystallized the non-phosphorylated/inactive form of the ALK kinase domain in complex with PHA-E429, TAE684 inhibitors and ATP observing the same protein structure. Surprisingly, intramolecular interactions between the N-terminal β-sheet and the DFG helix are responsible for ALK autoinhibition mechanism, this is different from what happens in the IR inactive conformation (Bossi, Saccardo et al. 2010). Intramolecular interactions are in fact typical of NRTK for locking of the catalytic domain in an inactive conformation (Schindler, Sicheri et al. 1999; Xu, Doshi et al. 1999). In particular, in the crystallized ALK structure, the DFG motif is connected to the N-terminal β-sheet through an hydrogen bond between Y1278 and C1097 residues. The α-C helix needs to shift toward the C-terminal lobe to properly position the catalytic residues during the protein activation. So, the link between the DFG and the N-terminal β-sheet is disrupted and phosphorylation of the three main tyrosine inside the A-loop and its release to adopt a fully active conformation can follow (Bossi, Saccardo et al. 2010). The unphosphorylated ALK revealed an A-loop DFG motif more similar to the "DFG-in" conformation (A-loop open) observed in active IGF1RK/IRK structure, rather than the "DFG-out" (A-loop closed) conformation observed in the inactive structures. The important Lys-Glu salt bridge,

observed in active kinase conformations, is present in the unphosphorylated ALK structure but finally the protein results inactive due to the restricted lobe closure and to the obstruction of the peptide binding site (Lee, Jia et al. 2010).

Fig. 4_ Domain structure of human ALK.

The extracellular region comprises two MAM domains (green) separated by an LDL-A domain (purple) and glycine-rich domain (G-rich, in blue). The transmembrane (TM)-spanning domain connects the N-terminal region with the intracellular tyrosine kinase domain (PTK, in red). Corresponding amino acids for each domain are showed. Figure adapted from (Lee, Jia et al 2010.).

ALK FULL LENGTH IN CANCER

ALK is involved in oncogenesis in both non-hematopoietic and hematopoietic malignancies. The full length form of ALK is expressed in different type of cancers including glioblastoma (Dirks, Fahnrich et al. 2002; Lu, Jong et al. 2005), breast cancer (Powers, Aigner et al. 2002), neuroblastoma (Lamant, Pulford et al. 2000; Osajima-Hakomori, Miyake et al. 2005), Ewing sarcoma (Zoubek, Simonitsch et al. 1995), retinoblastoma (Rassidakis, Lai et al. 2004), diffuse large B-cell lymphoma (Delsol, Lamant et al. 1997) and melanoma (Czubayko, Schulte et al. 1996). Apart from neuroblastoma, the pathogenic role of ALK in these tumours must be elucidated yet (Webb, Slavish et al. 2009).

Glioblastoma multiform is the most common highly aggressive human brain cancer. ALK overexpression in both glioblastoma cell lines and tumour cells together with the involvement of the ALK-PTN signalling pathway in the glioblastoma cell survival, seems to confirm a kinase involvement in the malignance even in the absence of ALK tyrosine phosphorylation in tumours (Dirks, Fahnrich et al. 2002; Powers, Aigner et al. 2002). Moreover ALK depletion in mice reduces tumour growth giving an advance in survival as a result of increased apoptosis (Tartari, Scapozza et al. 2011).

Neuroblastoma is the most common pediatric solid tumour. ALK full length is expressed in almost all primary neuroblastomas and neuroblastoma cell lines with ALK gene amplification in 10% cases (Lamant, Pulford et al. 2000; Dirks, Fahnrich et al. 2002; Osajima-Hakomori, Miyake et al. 2005). The demonstration of an ALK role in the pathogenesis of neuroblastoma, despite the lack of the endogenous tyrosine phosphorylation in neuroblastoma cell lines, derives from the observation of the ALK/Shc-C signalling involvement in tumour transformation. In particular, ALK is associated with the PTB domain of Shc-C in three neuroblastoma cell lines (NB-39-nu, Nagai and NB-1) showing ALK gene amplification and the presence of a constitutively activated ALK receptor. Shc proteins (Shc-A, Shc-B, Shc-C) play an important role in cellular signal transduction, they bind phosphotyrosine residues of different activated receptor tyrosine kinases (Miyake, Hakomori et al. 2002). Suppression of ALK activity in neuroblastoma cells reduces the phosphorylation of Shc-C, MAPKs and Akt, downstream targets of ALK, and induces apoptosis (Osajima-Hakomori, Miyake et al. 2005). Actually, 20 activating ALK mutations were

found in both familiar and sporadic cases of neuroblastoma: T1081I and D1091N in the juxtamembrane domain; G1128A in the P-loop; T1151M in the kinase domain; M1151R and I1171N in the C-helix; F1174I, F1174C, F1174V, F1174L at the end of the C-helix; R1192P in the β4-strand; A1234T, F1245T, F1245I, F1245L, F1245C, I1250T in the catalytic loop; R1275L, R1275Q and Y1278S in the A-loop.

ALK FUSION PROTEINS IN CANCER

Chromosomal rearrangements involving *alk* gene and producing oncogenic fusion proteins have been found in several tumours. Up to now 17 ALK different fusion proteins have been discovered: NPM (nucleophosmin) (Pulford, Lamant et al. 1997; Falini, Bigerna et al. 1998; Kadin and Morris 1998; Jager, Hahne et al. 2005), ATIC (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase) (Ma, Cools et al. 2000; Trinei, Lanfrancone et al. 2000), ALO17 (ALK lymphoma oligomerization partner on chromosome 17) (Cools, Wlodarska et al. 2002), CARS (cysteinyl-tRNA synthetase) (Cools, Wlodarska et al. 2002; Debelenko, Arthur et al. 2003), CTLC (clathrin heavy chain) (Touriol, Greenland et al. 2000; Gascoyne, Lamant et al. 2003), MYH-9 (non-muscle myosin heavy chain) (Lamant, Gascoyne et al. 2003), MSN (meosin) (Tort, Pinyol et al. 2001; Tort, Campo et al. 2004), TFG (TRK-fused gene) (Hernandez, Pinyol et al. 1999; Rosenwald, Ott et al. 1999), TPM3 (tropomyosin 3) (Lamant, Dastugue et al. 1999), TPM4 (tropomyosin 4) (Meech, McGavran et al. 2001), RANBP2 (Ran-binding protein 2) (Ma, Hill et al. 2003), SEC31L1 (Panagopoulos, Nilsson et al. 2006), EML4 (Soda, Takada et al. 2008; Martelli, Sozzi et al. 2009), KIF5B (Takeuchi, Choi et al. 2009) and SQSTM1 (sequestosome 1) (Takeuchi, Soda et al. 2011) (**Fig 5**). The fusions interrupt the alk gene at 2p23. Except for MSN/ALK and MYH-9/ALK, all new proteins contain the same ALK portion with the entire cytoplasmic region. The fusion partner at the N-terminal is usually widely expressed in normal cells and controls the chimeric protein expression and localization. The ALK-partner has an oligomerization domain which mediates constitutive self association of the ALK fusion causing constitutive activation of the kinase domain (Webb, Slavish et al. 2009).

So, the main ALK fusion proteins present in cancer are:

- The **ATIC**/ALK protein is present in about 4% of ALK-positive ALCL cases resulting from an inversion on chromosome 2, inv (2)(p23q35). This chimeric protein has a molecular weight of 96 kDa and has a cytoplasmic localization. (Wlodarska, De Wolf-Peeters et al. 1998; Colleoni, Bridge et al. 2000; Ma, Cools et al. 2000; Trinei, Lanfrancone et al. 2000). ATIC is a constitutively expressed, bifunctional, homodimeric enzyme that catalyzes the penultimate and final enzymatic steps of the purine-nucleotide synthesis pathway. ATIC/ALK is a constitutive activated tyrosine kinase, it leads to the activation of cytoplasmic signal transduction pathways, and to transformation of rodent fibroblasts (Ma, Cools et al. 2000; Trinei, Lanfrancone et al. 2000).

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- **CTLC**/ALK fusion is present in about 1% of ALK positive ALCL cases and results from the t(2;17)(p23;q23) chromosomal translocation (Touriol, Greenland et al. 2000; Cools, Wlodarska et al. 2002). CTLC/ALK has a molecular weight of approximately 220-250 kDa, and demonstrates a granular cytoplasmic staining pattern reflecting binding of the fusion protein to clathrin coated vesicles, and constitutive kinase activity (Touriol, Greenland et al. 2000)

- The **MSN**/ALK fusion protein results from a t(X;2)(q11-12;p23) chromosomal translocation. This fusion shows a distinctive membrane-restricted immunostaining pattern (Tort, Pinyol et al. 2001). MSN/ALK has a molecular weight of 125 kDa and possesses kinase activity as anti-phosphotyrosine staining was co-localized with MSN/ALK at the membrane. The unique cell membrane staining pattern is believed to reflect the association of MSN with membrane proteins. The ALK breakpoint in this chimeric MSN/ALK occurred in the exonic sequence coding the juxtamembrane region of ALK, which is 17 bp downstream of most other ALK breakpoints. It is likely that MSN/ALK can dimerize due to the binding of MSN to membrane proteins (Tort, Pinyol et al. 2001).

- Interestingly, **TFG** is involved in three different fusions with ALK, TFG/ALK(S), TFG/ALK(L) and TFG/ALK(XL) arising from breakpoints at introns 3, 4, and 5 of the TFG gene on chromosome 3q21, The resulting proteins molecular weights is

of 85, 97 and 113 kDa, respectively (Hernandez, Pinyol et al. 1999; Hernandez, Bea et al. 2002; Tort, Campo et al. 2004). TFG was first detected fused to the NTRK1 gene in thyroid papillary carcinoma, now it is known that this protein is constitutively expressed in many tissues including normal and neoplastic haematopoietic tissues (Greco, Mariani et al. 1995), while TFG/ALK expression is restricted to the cytoplasm. Has been demonstrated that TFG/ALK protein binds to the signalling proteins Grb2, Shc and PLC-γ being able to transform NIH3T3 fibroblasts *in vitro* (Tartari, Scapozza et al. 2011).

- **RANBP2**/ALK is a 1,430 amino acid chimeric protein deriving from chromosomal translocation t(2,2)(p23;q11-13) or inversion inv(2)(p23;q11-13). RANBP2 is a nucleopore protein of 358 kDa localized at the cytoplasmic side of the nuclear pore complex and has probably a role in nuclear-cytoplasmic trafficking (Mahajan, Delphin et al. 1997) Myofibroblasts expressing RANBP2/ALK exhibit nuclear membrane associated ALK staining that is unique compared to the subcellular localization observed with other ALK fusions in inflammatory myofibroblastic tumours (IMT). This is supposed to be caused by the hetero-association of the fusion with normal RANBP2 at the nuclear pore (Ma, Hill et al. 2003).

- The **SEC31L1**/ALK hybrid protein, resulting from the chromosomal translocation t(2;4)(p23;q21) is present in a subgroup of intra-abdominal inflammatory myofibroblastic tumours of young man (Panagopoulos, Nilsson et al. 2006). The SEC31L1 protein is ubiquitously and abundantly expressed, it is component of the COPII coat complex, which mediates transport from the endoplasmatic reticulum to the Golgi machinery. In fact SEC31L1 is present in the cells into vesicular structures that are scattered throughout the cell but are concentrated in the perinuclear region (Shugrue, Kolen et al. 1999; Tang, Zhang et al. 2000).

- **EML4**/ALK is the result of the chromosome inversion inv(2)(p21,p23) which produces two different proteins of 90 and 120 kDa depending on the breakpoint within EML4. This hybrid protein has been observed in 3-5% of non small cell lung cancer (NSCLC) cases (Soda, Takada et al. 2008; Martelli, Sozzi et al. 2009) and subsets of breast and colorectal cancer (Lin, Li et al. 2009).

- The **KIF5B**/ALK fusion protein, producing by the t(2,10)(p23,p11) involving intron 24 of KIF5B and intron 19 of ALK, has also been recently observed in NSCLC cases. The fusion protein is localized at the periphery of the cytoplasm, this could reflect transport of KIF5B-ALK along microtubules (Takeuchi, Choi et al. 2009).

- The **SQSTM1**/ALK fusion protein is produced by a translocation t(2;5)(p23.1,q35.3), in particular the exon 5 of SQSTM1 is in-frame fused to the exon 20 of ALK. This protein has recently been observed in a case of large B-cell lymphoma with a cytoplasmatic distribution. SQSTM1 is an ubiquitin binding protein that is associated with oxidative stress, cell signalling, and autophagy (Takeuchi,Soda et al. 2011).

Translocation	Frequency in ALK+ lymphoma	Frequency in NSCLC	Occurrence in IMT	Localization	
t(2;5)(p23;q35)	~75%	–	–	N/C	NPM ALK
t(1;2)(q25;p23)	~18%	–	+	C	TMP3 ALK
inv(2)(p23;q35)	~2%	–	–	C	ATIC ALK
t(2;17)(p23;q23)	~2%	–	+	C	CLTC ALK
t(2;3)(p23;q21)	~1%	–	–	C	TFG_L ALK
t(2;3)(p23;q21)	~1%	–	–	C	TFG_S ALK
t(2;19)(p23;p13.1)	<1%	–	+	C	TPM4 ALK
t(2;X)(p23;q11-12)	<1%	–	–	CM	MSN ALK
t(2;2)(p23;q11-13)? or inv(2)(p23;q11-13)?	–	–	+	NM	RanBP2 ALK
inv(2)(p21;p23)	–	3 ÷ 7%	–	C	EML4 ALK

Fig. 5_ Schematic representation of the most frequent ALK fusion proteins.
The figure shows the translocation leading to each fusion protein, its frequency in ALK positive lymphoma and the cellular localization. N: nuclear, C: cytoplasmic, CM: cell membrane, NM: nuclear membrane. From (Ardini, Magnaghi et al. 2010).

HAEMATOLOGICAL DISEASES INVOLVING ALK FUSION PROTEINS

Considering haematological malignancies, ALK rearrangements have been found only in lymphoma, in particular in ALCL and in ALK-positive diffuse large B-cell lymphoma (DLBCL).

ALCL

The Anaplastic Large Cell Lymphoma accounts for approximately 5% of all human non-Hodgkin's lymphomas (NHLs), it is predominant in young patients (83%) comprising 30-40% of pediatric large-cell lymphomas (Sandlund, Pui et al. 1994; Drexler, Gignac et al. 2000). ALCL is an aggressive tumour usually discovered at stage III-IV, it is associated with systemic symptoms and extranodal involvement especially of skin and bone (Stein, Foss et al. 2000; Morris, Xue et al. 2001).

60-80% of ALCLs cases are ALK-positive, cells are CD30-positive and without expression of both T and B-cell markers (Benharroch, Meguerian-Bedoyan et al. 1998; Kinney and Kadin 1999; Morris, Xue et al. 2001). ALK-positive lymphomas, commonly referred to as "ALKomas", are classified as ALK-positive ALCL in the WHO's (World Health Organization) classification of ALCL (Webb, Slavish et al. 2009).

NPM/ALK is the most common ALK hybrid protein in NHL, in 5-10% of cases, and it is present in approximately 75-80% of all ALK-positive Anaplastic Large Cell Lymphoma (ALCLs) (Mason, Bastard et al. 1990; Kadin and Morris 1998). The other ALK fusion proteins found in patients affected by ALCL are: ATIC/ALK, CTL/ALK, MSN/ALK, MYH9/ALK, TGF/ALK, TPM3/ALK, TPM4/ALK (Tartari, Scapozza et al. 2011).

The PF-2341066 (crizotinib) is now under investigation for the ALCL ALK positive treatment, clinical studies are ongoing. Two patients, who had relapsed ALK-positive ALCL, showed complete and persisted response under crizotinib treatment (Gambacorti-Passerini et al, 2011).

DLBCL

DLBCL is a rare form of B-cell NHL expressing CLTC/ALK, NPM/ALK, MYH9/ALK or SQSTM1/ALK fusion proteins (Takeuchi, Soda et al. 2011; Chikatsu, Kojima et al. 2003; De Paepe, Baens et al. 2003; Gascoyne, Lamant et al. 2003;

Lamant, Gascoyne et al. 2003; Onciu, Behm et al. 2003). This is an aggressive lymphoma with a poor response to chemotherapy, cells lack CD30 expression and show intra-cytoplasmic IgA and the plasma cell-associated marker (p63 expression) (Webb, Slavish et al. 2009).

ALK-positive systemic histocytosis

TPM3/ALK fusion protein has been found in a case of histocytosis in early infancy. Interestingly, other two cases of this disease exhibited ALK immunoreactivity. In this case, the ALK-transformed cell of origin seems to be of macrophage or dentritic cell lineage (Chan, Lamant et al. 2008).

NPM/ALK

NPM/ALK is the first identified ALK fusion protein. The chromosomal translocation t(2,5) fuses the *npm* nucleolar phosphoprotein gene on chromosome 5q35 to the *alk* protein tyrosine kinase gene on chromosome 2p23 (**Fig 6**). The chromosomal breakpoint involves the intron 4 of *npm* and the intron 16 of *alk*. As result, the N-terminal region of NPM is bound to the catalytic domain of ALK and is able to control its expression and localization (Morris, Kirstein et al. 1994; Kadin and Morris 1998).

NPM is a multifunctional protein involved in the shuttling of pre-ribosome subunits from the nucleus to the cytoplasm during ribosome biogenesis, in regulation of cell division, DNA repair, transcription and genomic stability (Okuwaki 2008). NPM has a nucleolar localization signal and a dimerization domain which brings to protein oligomerization resulting in autophosphorylation and activation of ALK kinase domain. NPM is essential for the transforming ability of NPM/ALK, in fact mutation of the ATP-binding site (K219R) produces a kinase dead version of NPM/ALK, not able to induce transformation (Bischof, Pulford et al. 1997); while, deletion of the NPM dimerization domain fails to transform cells (Ladanyi and Cavalchire 1996).

NPM/ALK expression is responsible for oncogenic transformation both *in vitro* and *in vivo*. *In vitro* the fusion protein induces growth factor independence proliferation in BaF3 cells and PC12 neural cells; moreover, murine and rodent fibroblasts show a transformed phenotype in its presence (Fujimoto, Shiota et al. 1996; Bischof, Pulford et al. 1997; Mason, Pulford et al. 1998; Chiarle, Gong et al.

2003). *In vivo* lethally irradiated BALB/cByJ mice transfected with bone marrow derived-cells expressing NPM/ALK develop B-cell lymphomas within 4, 6 months (Kuefer, Look et al. 1997). Mice with chimeric fusion expression limited to T cells develop thymic lymphomas and plasma cell neoplasms from 5 week of age (Chiarle, Gong et al. 2003); while transplanted retrovirally transduced bone marrow over-expressing NMP/ALK into IL-9 transgenic mice develop T cell lymphoblastic lymphomas, plasmacytomas and plasmablastic/DBLCL after 20 weeks (Lange, Uckert et al. 2003).

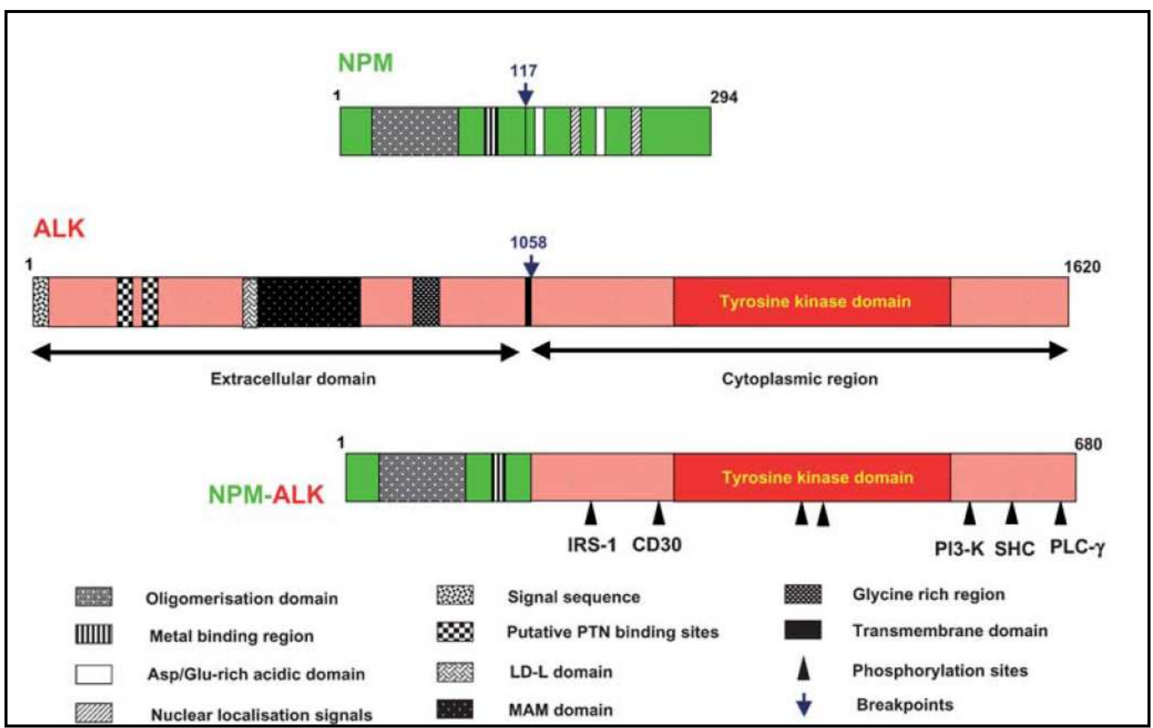


Fig 6_ Full-length ALK and NPM/ALK fusion protein structure

The domains and the binding sites of various important molecules of the signalling pathways are indicated. In blue is the breakpoint. Figure. from (Pulford, Lamant et al. 2004).

ONCOGENIC SIGNALLING MEDIATED BY ALK-FUSION PROTEINS

NPM/ALK is able to activate proliferative pathways such as RAS/MAPK, PLCγ and JAK (Janus kinase)/STAT pathways directly or via adaptor proteins. Furthermore, NPM/ALK can protect cells from apoptosis mediated by PI3K/Akt activated pathway, or by inducing transcription of genes including Bcl-2 and Bcl-xL. These pathways are typically up-regulated in transformed cell lines (**Fig 7**).

PLCγ directly interacts with NPM/ALK via its SH2 (Src homology 2) domain. Has been demonstrated that activation of the PLCγ pathway is essential for transformation in transfected cell through the serine/threonine protein kinase C (PKC). Y664 of NPM/ALK, corresponding to Y1604 in the ALK full length, is the docking site for PLCγ (Bai, Dieter et al. 1998).

The **PI3K** binding to NPM/ALK, through the PI3K SH2 and SH3 domains, leads to phosphorylation/activation of the PKB/Akt pathway and regulation of subsequent downstream signalling events. An indirect interaction between NPM/ALK and PI3K involving adaptor molecules is also present. This pathway is important in the regulation of the anti-apoptotic signalling and is critical for cell transformation mediated by NPM/ALK (Bai, Ouyang et al. 2000; Slupianek, Nieborowska-Skorska et al. 2001; Polgar, Leisser et al. 2005). Active PKB/Akt is able to phosphorylate FOXO3a (forkhead box O 3a) and mTOR (mammalian target of rapamycin). FOXO3a is a transcription factor, when phosphorylated it remains in the cytoplasm with a consequent down-regulation of target genes as cyclin D2, Bin-1 and $p27^{kip1}$ controlling cell-cycle arrest (Gu, Tothova et al. 2004; Rassidakis, Feretzaki et al. 2005). mTOR phosphorylation by the PI3K/PKB/Akt pathway results in its activation in cells (Palmer, Vernersson et al. 2009).

The **RAS/MAPK pathway** involves IRS-1, Shc and Grb2 (growth-factor-receptor-bound protein 2) adaptor proteins. Interaction with IRS-1 and Shc is not essential for transformation, in fact NPM/ALK mutants not able to interact with Shc and IRS-1 are still able to transform NIH 3T3 cells (Fujimoto, Shiota et al. 1996). NPM/ALK is also able to directly activate MEK inducing cell proliferation (Crockett, Lin et al. 2004; Marzec, Kasprzycka et al. 2007).

NPM/ALK is also able to phosphorylate STAT3 activating the **JAK/STAT3 pathway**, in fact inactivation of the fusion protein by ALK inhibitors reduces STAT3

phosphorylation (Marzec, Kasprzycka et al. 2005; Wan, Albom et al. 2006; Galkin, Melnick et al. 2007). NPM/ALK binds to JAK3, the receptor-associated tyrosine kinase responsible for STAT3 activation, and the inhibition of JAK3 results in a reduction of STAT3 activation and in an increased of cellular apoptosis. So JAK3 activity is strongly associated with ALK expression and STAT3 phosphorylation *in vivo* (Amin, Medeiros et al. 2003; Lai, Rassidakis et al. 2005; Shi, Franko et al. 2006). Down-regulation of active STAT3 in ALK positive cells leads to an increase in apoptosis and to cell cycle arrest. STAT3 has also a role in NPM/ALK-induced tumour growth *in vivo* (Amin, Medeiros et al. 2003; Chiarle, Simmons et al. 2005; Marzec, Kasprzycka et al. 2005; Han, Amin et al. 2006; Qiu, Lai et al. 2006). Methylation in ALK-positive ALCL cells is responsible for loss of the JAK/STAT pathway negative regulator Shp1 (SH2 domain-containing phosphatise 1). In fact, Shp1 is able to inactivate the JAK/STAT pathway and block cell-cycle progression (Khoury, Rassidakis et al. 2004; Han, Amin et al. 2006). Moreover, protein phosphatase 2A overexpression has been observed in ALK-positive ALCL. The phosphatase is a STAT3-interacting protein necessary for sustained STAT3 phosphorylation (Han, Amin et al. 2006; Zhang, Wang et al. 2007).

NPM/ALK has also been shown to stimulate constitutive STAT5, another member of the STAT family, phosphorylation and activation in transformed cells (Nieborowska-Skorska, Slupianek et al. 2001). Expression of dominant negative STAT5 by retroviral infection in NPM/ALK expressing cells inhibited the antiapoptotic signalling mediated by NPM/ALK, as well as proliferation and clonogenicity. In addition, dominant negative-STAT5 (DN-STAT5) also prolonged survival of SCID mice injected with NPM/ALK positive-cells. NPM/ALK has also been shown to bind to and activate JAK2, leading to proliferative and anti-apoptotic signals. Inhibition of JAK2 by AG490 inhibited STAT5 activation and induced apoptosis and growth inhibition (Ruchatz, Coluccia et al. 2003).

The **Bcl-2** family members are important modulators of mitochondrially initiated apoptosis (Reed, Jurgensmeier et al. 1998). This family contains both pro-survival (Bcl-2, Bcl-xL, Mcl-1) and pro-apoptotic (Bad, Bak, Bax) factors. It has been demonstrated that NPM/ALK blocks the activation of caspase-3 by preventing the cytosolic accumulation of cytocrome-c (Ergin, Denning et al. 2001). In addition, transcriptional induction of Bcl-xL is elicited by NPM/ALK through the constitutive activation of the JAK3/STAT3 pathway, protecting cells from drug-induced apoptosis (Zamo, Chiarle et al. 2002; Amin, Medeiros et al. 2003; Ruchatz, Coluccia et al.

2003). Furthermore, NPM/ALK kinase activity was required to promote Bcl-xL expression and its protective effect on mitochondrial homeostasis. Down-regulation of Bcl-xL significantly reduced the anti-apoptotic potential of NPM/ALK in both transformed murine Ba/F3 cells and human ALCL-derived Karpas 299 cells (Coluccia, Perego et al. 2004).

NIPA (nuclear interacting partner of ALK) is a novel downstream target of NPM/ALK, this protein targets nuclear cyclin B1 for ubiquitination during interphase regulating the mitotic entry. NIPA has an anti-apoptotic role in NPM/ALK cells, in fact its overexpression is able to protect Ba/F3 cells from apoptosis (Ouyang, Bai et al. 2003).

Several RNA/DNA-binding proteins coimmunoprecipitate with NPM/ALK. In particular, the multifunctional polypyrimidime tract binding protein associated splicing factor **PSF** has been found to be a novel NPM/ALK binding protein and substrate. Association of PSF with active NPM/ALK was confirmed in ALCL derived cell lines and patient samples. The bindind leads to PSF Tyr293 phosphorylation and to its delocalization to the cytoplasm inhibiting the PSF transcriptional repressor functions (Galietta, Gunby et al 2007).

Recently an increased of **SHH** (sonic hedghog) expression in ALK-positive ALCL has been discovered. This over-expression seems to be dependent on NPM/ALK-induced PI3K activity, in fact PI3K inhibition leads to a concentration-dependent decrease of SHH protein levels. SHH regulates cell viability in ALK-positive ALCL cell lines (Singh, Cho-Vega et al. 2009).

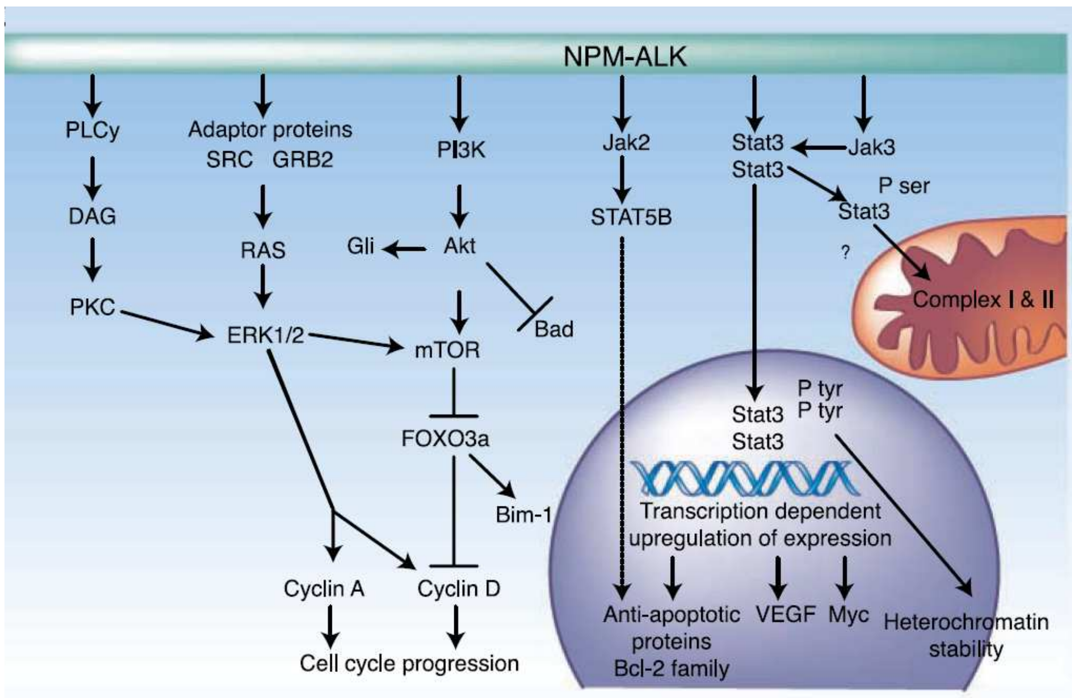


Fig 7_NPM/ALK signalling transduction pathways

NPM/ALK activation induces transformation via different signalling pathways leading to apoptosis-resistance and cellular proliferation. Figure from (Mossé, Wood et al. 2009).

ALK AS THERAPEUTIC TARGET IN CANCER

ALCL, the main haematological malignance expressing ALK, is currently treated with combination chemotherapy with success in 60-70% of cases and ALK-positive ALCL has a better prognosis than ALK-negative ALCL or other T-cell NHLs. In fact ALK is a good candidate for the development of targeted treatment because of a lack of widely expression in normal-adult tissues. So blocking ALK function should not give important toxic effects (Chiarle, Voena et al. 2008).

Potential strategies for targeting ALK includes immunotherapy, gene silencing, inhibition of downstream signalling pathways and direct inhibition of its catalytic activity through small-molecules inhibitors. All these strategies aim to obtain a specific treatment reducing side effects in patients, different from what happens with the conventional cytotoxic chemotherapeutics (Coluccia, Gunby et al. 2005) (**Fig 8**).

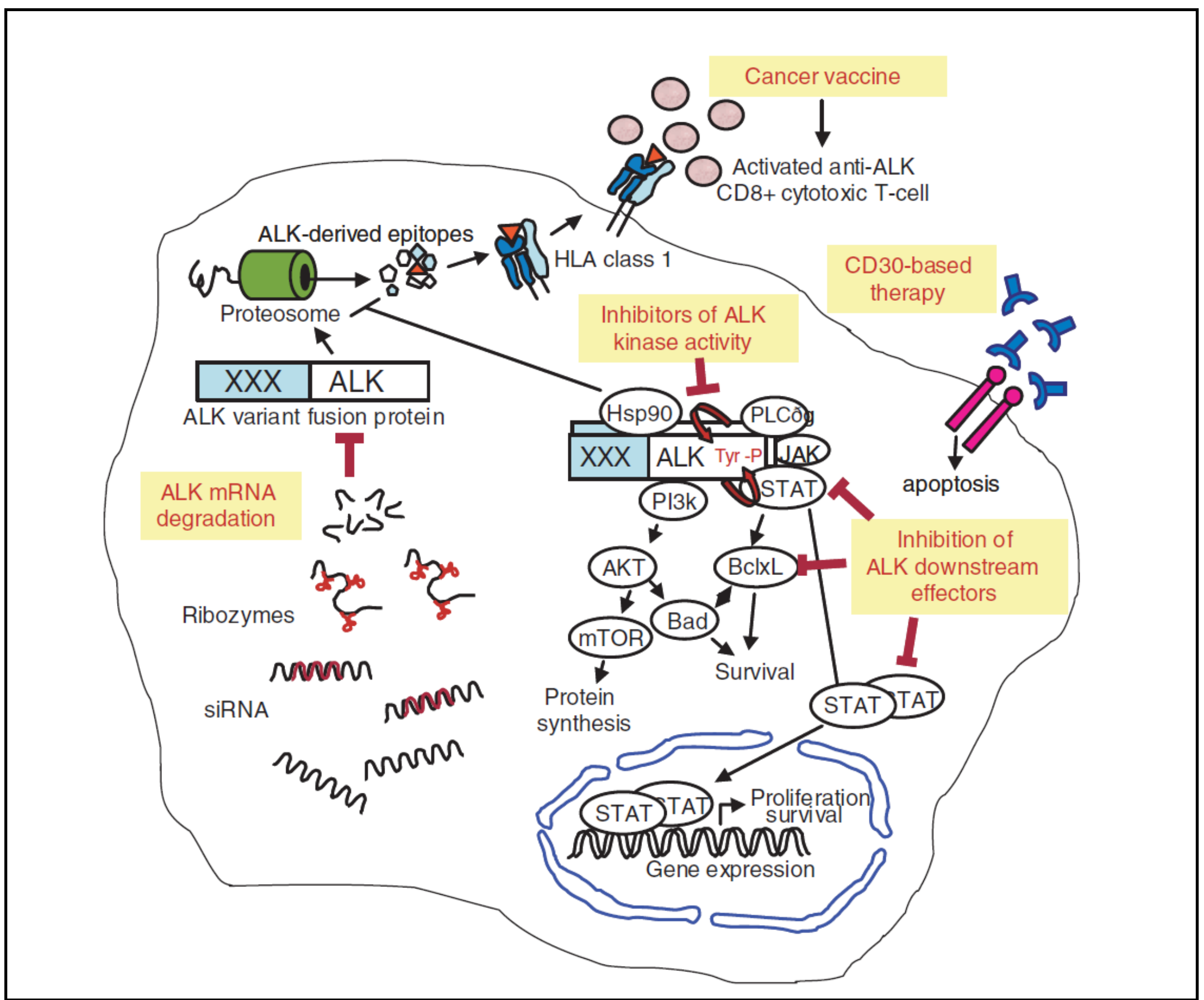


Fig 8_ Potential therapeutic approaches against ALK.

The different strategies for ALK targeting, in the yellow boxes, are summarized in this scheme. HLA: Human leukocyte antigen; Hsp: Heat-shock protein; JAK: Janus kinase; mTOR: Mamallian target of rapamycin; PI3K: Phosphatidylinositol 3-kinase; PLC: Phospholipase C; STAT: Signal transducer and activator of transcription. From (Coluccia, Gunby et al. 2005).

IMMUNOTHERAPY

Cancer vaccination is the specific activation of the T cell-mediated immune response against specific antigens expressed by the tumour cells to enhance anti-tumour responses or to gain the complete eradication of cancer cells (Berzofsky, Ahlers et al. 2004; Panelli, Wang et al. 2004). Success of this approach depends on the availability of suitable tumour antigens. ALK could be a target of anti-tumour vaccination because ALK-positive cells are completely dependent on ALK activity for survival and proliferation. Moreover ALK normal expression is restricted to some neuronal cells with low expression, compared with high expression in cancer cells,

leading to specificity of the vaccination. Finally ALK is spontaneously immunogenic in ALCL patients eliciting both antibody and T cell-mediated cytotoxic responses (Pulford, Falini et al. 2000; Ait-Tahar, Cerundolo et al. 2006; Piva, Chiarle et al. 2006). Therefore, vaccination in combination with chemotherapy could improve ALK-related diseases treatment.

GENE SILENCING (RNAi)

Small interfering RNA (siRNA) is an alternative approach to obtain ALK inhibition through gene expression silencing. siRNA are small double stranded RNA sequences that leads to the target mRNA degradation in a sequence specific manner. ALK-specific siRNA duplexes and selective ribozyme were used to obtain the ablation of the ALK protein: protein expression decrease leads to cell-cycle arrest followed by apoptosis both *in vitro* and *in vivo* (Piva, Chiarle et al. 2006). Merkel et al. (Merkel, Hamacher et al. 2010) studied the expression of miRNA, endogen small non-coding RNAs acting as siRNA in regulating gene expression. In addition, miR-10 is down-regulated in primary ALCL formalin fixed, paraffin-embedded tissue samples, in ALCL mouse model and in ALCL cell lines and is able to reduce proliferation in ALK-positive cell lines after reintroduction. However this strategy needs more studies to be useful in clinic.

INHIBITION OF r-ALK DOWNSTREAM SIGNALLING PATHWAYS

This strategy uses pathways-targeted small-molecule inhibitors or antisense molecular strategies to block downstream signalling that are constitutively activated in ALK positive cells and leads to transformation, proliferation and cell survival. Inhibitors of PI3K are present, like wortmannin, a fungal metabolite (Bai, Ouyang et al. 2000) and LY-294002, a flavoniod derivative (Coluccia, Gunby et al. 2005). Also inhibitors of the JAK/STAT pathways and of the Bcl-X_L protein have been discovered. In particular S3I-201 that directly deactivates STAT3 by inhibiting DNA binding and STAT3 complex formation (Siddiquee, Zhang et al. 2007). For ALK downstream targets inhibition, also siRNA could be used; targeting STAT3 with specific ASO in xenograft models in mice leads to cell-cycle arrest and apoptosis of ALCL ALK-positive cells. ASO are antisense oligonucleotide similar to RNAi molecules but simpler and not naturally produced by cells, the principle is the same:

they stick to the mRNA message molecule and prevent the cell from using it to build proteins (Chiarle, Simmons et al. 2005).

ALK downstream signalling pathways inhibitor do not represent a good choice for therapy because of the presence of a big redundancy of signal transduction pathways in each tumour, so each patient should need a specific inhibitors cocktail (Barreca, Lasorsa et al. 2011).

ALK SMALL MOLECULES INHIBITORS

After the identification of constitutively activated forms of ALK in different types of tumours, both as fusion proteins and as mutated ALK forms, the attention has focused on the development of small molecules targeting the protein kinase activity in order to abolish the ALK dependent cancer cell growth (Ardini, Magnaghi et al. 2010). The designed kinase inhibitors are directed against the ATP-binding site of the catalytic domain, which is highly conserved in kinases, to obtain a successful ALK inhibition. Targeting different kinase conformation and enzyme specific lipophilic pockets, whose accessibility is dependent on the gatekeeper residue, is a way to solve the problem of inhibitor selectivity. Most developed molecules bind closed to the ATP-binding site using a part of their scaffold to mimic the binding of the adenine moiety of ATP, these compounds compete with the endogenous ATP for binding (Ardini, Magnaghi et al. 2010 ; Madhusudan and Ganesan 2004).

Nowadays, different small molecule inhibitors of TKs including ABL (Abelson proto-onocogene), EGFR, BRAF, VEGF, PDGF have been studied. Knowledge about kinase structure, regulation and activation of the kinase, derived also from crystal structure, is very important for inhibitors production. Gleevec (Imatinib, STI571, Novartis) is the first clinically approved TK inhibitors, it represents a paradigm for small molecules inhibitors development (Tartari, Scapozza et al. 2011). Imatinib has been developed thanks to structural characterization of the ABL kinase domain, it binds to the inactive form of the kinase domain of ABL responsible for the chronic myeloid leukemia (CML), as well as c-KIT and the PDGF receptor blocking the kinase activity (Buchdunger, Zimmermann et al. 1996; Druker, Tamura et al. 1996; Schindler, Bornmann et al. 2000). Imatinib is actually used in the treatment of CML (Kantarjian, Sawyers et al. 2002; Talpaz, Silver et al. 2002). After Imatinib resistance and intolerance appeared, second generation BCR-ABL inhibitors have been produced: Sprycel (dasatinib) recently approved for clinical use and Tasigna

(nilotinib) recently approved for therapy of imatinib-resistant CML (Webb, Slavish et al. 2009).

Knowledge about protein structures is very important to understand the mechanism that regulates the kinase activity, this permits the development of specific and efficient inhibitors that can be used in clinic. Crystal structure of several tyrosine kinases such as Src/Abl and the IR has given great advantage in the study of new compounds, moreover sequence analysis of kinase domain of protein belonging to the same family allowed to design common structural features of a kinase domain.

Molecular modelling, producing a 3D-structure generated by homology with kinases of the same ALK family, has been very useful and has been used in the drug design process before the recently publishing of ALK crystal structure.

Natural ALK-inhibitors

Staurosporine is a natural compound inhibiting ALK with IC_{50} of 150nM in the presence of 30µM ATP in an ELISA kinase assay, and inhibiting ALK autophosphorylation in cells with an IC_{50} of 1µM (Gunby, Tartari et al. 2005) (**Fig 9**). Staurosporine is a potent inhibitor but without specificity, so derivatives as *7-hydroxystaurosporine (UCN-01)* have been produced to reduce toxicity (Wang, Fan et al. 1996) (**Fig 9**). UCN-01 has less activity on recombinant ALK KD, with an IC_{50} of 5 µM in presence of 30µM ATP (Gunby, Tartari et al. 2005). UCN-01 is used in phase I study in patients with refractory neoplasms and in phase II study in relapsed or refractory systemic ALCL and mature T-cell lymphomas (Sausville, Arbuck et al. 2001)

Other natural-derived compounds are *geldanamycin, 17-allylamino-17-demethoxygeldanamycin* and *herbimycin A* (Li and Morris 2008). These molecules inhibit ALK increasing the proteosome-mediated degradation of the ALK protein binding to heat shock protein 90 (HSP-90) (Turturro, Arnold et al. 2002; Bonvini, Dalla Rosa et al. 2004; Georgakis, Li et al. 2006).

Staurosporine-based compounds *CEP-14083* and *CEP-14513* (**Fig 9**) have been synthesized by Cephalon (Frazer, PA) and they have been identified by high-throughput screening. They show an enzymatic ALK IC_{50} <5nM and inhibition of tyrosine phosphorylation in cell with an IC_{50}<30nM (Wan, Albom et al. 2006). These pyrrolocarbazoles lead to G1 cell cycle arrest and apoptosis in ALCL NPM/ALK-positive cell lines inactivating ERK1/2, AKT and STAT3. These compounds are also

active against VEGFR2, TIE2 and DLK (Wan, Albom et al. 2006). The use *in vivo* of these inhibitor is limited because of their unfavourable physicochemical properties.

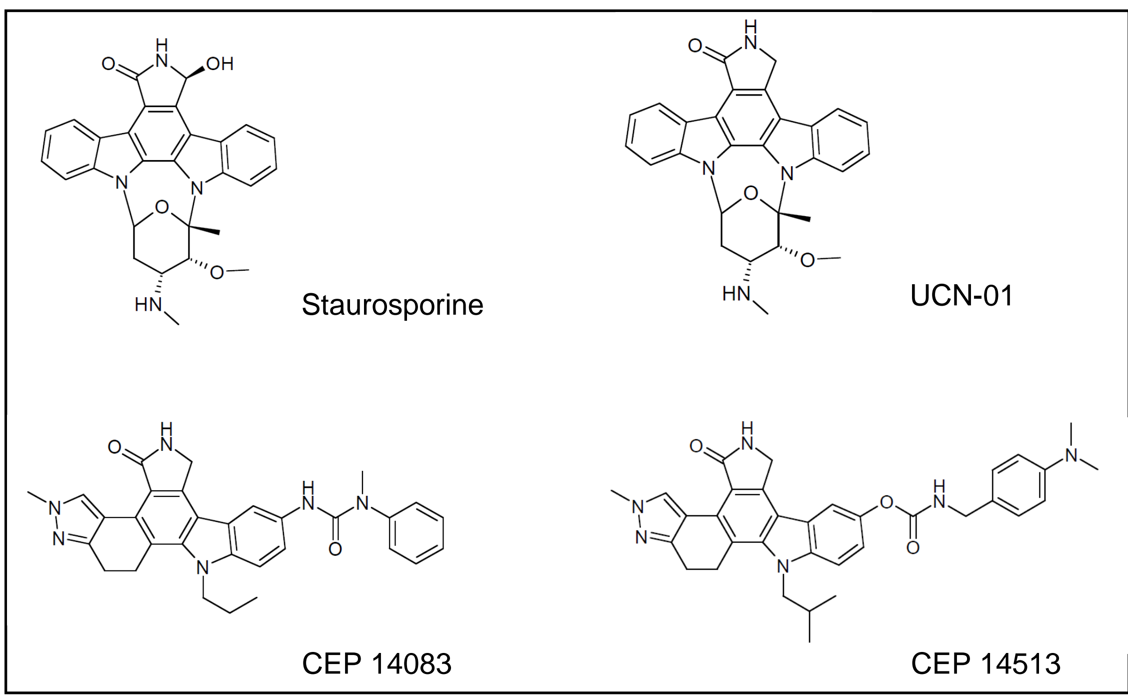


Fig 9_ Natural ALK inhibitors

Synthetic ALK inhibitors

Pyridone derivatives are potent ALK inhibitor, they include pyridine 14 and pyridine 15 (Li, Xue et al. 2006). **NVP-TAE684** (5-chloro-2,4-diaminophenylpyrimidine) (**Fig 10**) has been identified using a cellular screen of a kinase-directed small-molecule library to search for compounds cytotoxic to BaF3-NPM/ALK transfected cells. NVP-TAE684 exhibits a cellular IC_{50} of 3nM on transfected cells with low toxicity at 1μM on parental BaF3 cells and an IC_{50} of 2-5nM in ALCL ALK-positive cells inducing G1 cell cycle arrest and apoptosis. This inhibitor has a good oral bioavailability *in vivo* (60-70%) and it is able to inhibit the development of systemic Karpas-299 tumours (Galkin, Melnick et al. 2007). NVP-TAE684 has been designed using an ALK homology model based on the published crystal structure of the IR KD in an active conformation. TAE is not currently being tested as a clinical agent.

PF-2341066 (crizotinib) (**Fig 10**), a dichloro compound from Pfizer, is a dual ALK/MET inhibitor displaying inhibition of cellular phosphorylation with IC_{50} of 24nM for ALK and 11nM for MET. PF-2341066 is able to block cellular proliferation of NPM/ALK positive ALCL cells with an IC_{50} of 32nM in Karpas-299 and of 43nM in SU-DHL-1 cells, inducing G1 cell cycle arrest and apoptosis. Moreover, this inhibitor

gives tumour regression in a Karpas-299 human NPM/ALK ALCL cell line xenograft model (Christensen, Zou et al. 2007; Zou, Li et al. 2007). It has been demonstrated that PF-2341066 is able to improve survival in advanced ALK-positive NSCLC cases (Shaw, Yeap et al 2011). On August 26 2011, it has been approved, by the U. S. Food and Drug Administration (FDA), for the treatment of patients with locally advanced or metastatic NSCLC. This inhibitor has also showed interesting effects in ALK-positive lung cancer, IMT and ALCL. Crizotinib is now under study also in neuroblastoma, in particular for its activity against ALK F1174L mutations (Tartari, Scapozza et al. 2011).

CRL151104A (no structure available) is a third-generation pyridone compound ATP competitor. It shows an IC_{50} of 9.75nM *in vitro* in presence of 100μM of ATP and an IC_{50} <100nM, 2.5 μM *in vivo* for NPM/ALK positive and NPM/ALK negative cells respectively. CRL151104A is able to completely block cellular phosphorylation and is active against some ALK mutation in neuroblastoma including F1174L and R1275Q (Webb, Slavish et al. 2009).

WZ-5-126 (no structure available) is a potent ALK small molecules inhibitor, it displays an IC_{50} of 3.4nM *in vitro* and effects *in vivo* inhibiting the growth of two NSCLC cell lines ALK-positive (McDermott, Iafrate et al. 2008).

Other ALK inhibitor are **NMS-E628**, from Nerviano, and **AP-26113** from ARIAD laboratories. The first one is able to block proliferation and intracellular signalling activation of ALK-dependent cell lines, it's able to give complete tumour regression in SCID mice with Karpass-299 or in SR-786 xenografts. AP-26113 overcomes resistance against some ALK mutations (G1269S and L1196M) (Grande, Bolos et al. 2011). According to preclinical studies, AP-26113 is 10 fold more potent compared to PF-02341066 and displays activity in ALCL and NSCLC models (Tartari, Scapozza et al. 2011).

Recently Sakamoto et al. discovered **CH5424802** (**Fig 10**), a benzocarbazole derivative identified by high-throughput inhibitor screening against multiple cancer-related tyrosine kinase cancer as a potent and selective orally available ALK inhibitor. It displays an IC_{50} of 1.9nM *in vitro* and is able to prevent ALK autophosphorylation and consequent STAT3 and AKT phosphorylation in NCI-H2228 NSCLC ALK-positive cells. CH5424802 is also active against C1156Y and L1196M mutation forms of ALK. These molecule is now being investigated in phase I/II clinical trials for patients with ALK-positive NSCLC (Sakamoto, Tsukaguchi et al. 2011).

GSK1838705A (**Fig 10**) inhibits both ALK and the IGF receptor-1 (IGF-1R) with an enzymatic IC_{50} of 2nM and 0.5nM respectively (Sabbatini, Korenchuk et al. 2009).This inhibitor is active in ALK-positive cell lines, in mice bearing tumour xenografts and in tumour models leading to the complete tumour regression. The drug is not under clinical trial yet (Grande, Bolos et al. 2011).

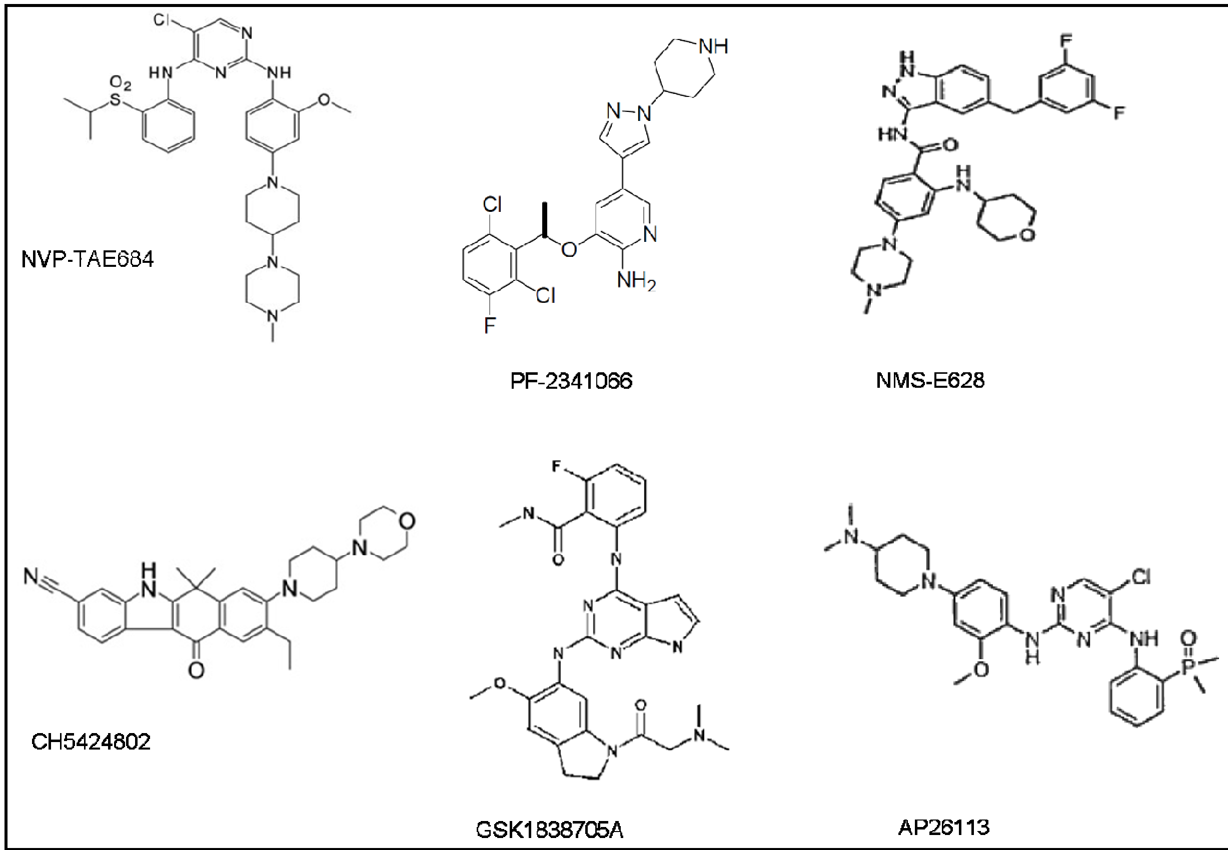


Fig 10_Synthetic ALK inhibitors

Actually a number of ALK inhibitors are under investigation, but any of them has yet been approved as anticancer agent for ALCL specific treatment. Acquired resistance to kinase inhibitors is a serious problem in long-term cancer treatment that has to be considered to obtain a good result in clinical treatment of ALCL. An example is given by CML treatment, where Imatinib is not efficient against BCR/ABL T315I gatekeeper mutant and new inhibitors as AP24534 have been developed thanks to the structural analysis of the kinase domain (O'Hare, Shakespeare et al. 2009). Recently C1156Y and L1196M mutations have been discovered conferring clinical resistance to Crizotinib in NSCLC patients while F1174L is resistant to the same drug in an IMT patient (Choi, Soda et al. 2010; Sasaki, Okuda et al. 2010). Therefore, in clinic a good ALK inhibitor overcoming mutations of the kinase domain

is still needed, and the structural study of the ALK kinase domain can accelerate this process giving information about binding between the protein and the drug. The information could be used to understand the interaction and to improve the small molecule efficiency or to create new ALK inhibitors.

AIM

The aim of this work is:

1. Production of different forms of the recombinant ALK kinase domain.
2. Purification and characterization of the recombinant ALK kinase domain; optimization of the purification procedure is necessary to achieve the following requirements:
   - milligrams of protein yield,
   - sample purity >95%,
   - sample concentration ≈10 mg/mL,
   - protein in the right secondary structure, all in single oligomeric species, not aggregated, enzymatically active and stable.
3. Structural studies of purified oncogenic protein through X-ray crystallography (in collaboration with Prof. Neil McDonald, Structural Biology Laboratory, Cancer Research UK London) and STD-NMR (in collaboration with Prof. Francesco Nicotra, Department of Biotechnology and Bioscience, University of Milano-Bicocca).

Structural determination could be very useful to study the possible interactions between ALK and specific inhibitors compounds that are actually on development.

Nowadays, there is not an efficient cure able to permanently eradicate the ALK+ disease yet. Therefore, results of this work could improve knowledge useful in the rational design of new specific drugs for treatment of the ALK+ anaplastic large cell lymphomas and other ALK+ tumours.

MATERIALS AND METHODS

Cloning of the ALK kinase domain into the pENTRY or pBacPAK expression vectors

- WT constructs:

The different kinase domain sequences are amplified by PCR (FastStart High Fidelity PCR System, Roche) using a full length NPM/ALK sequence cloned into a pcDNA3 as template. The PCR fragments are purified on column using the QIAquick PCR Purification Kit (Qiagen), controlled and quantified on agarose gel. pENTRY (Gateway entry vector, Invitrogen) / pBacPAK (a pBacPAK-His 3 modified by Prof Neil McDonald) expression vector is digested for 1 hour at 37℃, and dephosphorylated using the Calf Intestinal Phosphatase (CIP from New England Biolabs) for 1hour at 37℃. PCR fragments are digested and both fragments and expression vectors are purified on column (QIAquick PCR Purification Kit, Qiagen). Fragments and vectors are ligated for 4,5 hours at 16℃ using T4 DNA Ligase (New England Biolabs). The ligated DNA is used to transform Top10 cells. Bacterial colonies are grown in LB with Ampicillin 50 µg/mL. Miniprep is performed (QIAprep Spin Miniprep Kit, Qiagen), fragments right insertion is controlled by sequencing (performed by Eurofins MWG Operon and analyzed by Vector NTI Software). The amount of cloned DNA is increased (QIAprep Maxiprep Kit, Qiagen).

- L1196 mutant construct:

Site specific mutagenesis is performed (PFU ultra, Stratagene) using the pBacPAK ALK 6 as template. DNA is digested with DpnI (New England Biolabs) for 1 hour at 37℃, and used to transform Top10 cells. Bacterial colonies are grown in LB with Ampicillin 50 µg/mL. Miniprep is performed (QIAprep Spin Miniprep Kit, Qiagen), fragments right insertion is controlled by sequencing (performed by Eurofins MWG Operon and analyzed by Vector NTI Software). The amount of cloned DNA is increased (QIAprep Maxiprep Kit, Qiagen).

Generation of Baculovirus expressing r-GST-tagged ALK constructs

- GST-TEV r-ALK constructs:

Different r-ALK kinase domain sequences are cloned into a pDEST20 expression vector using the Bac-to-Bac Baculovirus expression system (Invitrogen). An LR site specific recombination reaction between the pENTRY containing the cloned r-ALK sequences and a Gateway destination vector (pDEST20) is performed. The recombinant vector is transposed into the Baculovirus shuttle vector, Bacmid, present in DH10Bac™ cells (using site-specific transposition properties of

the Tn7 transposone) to generate a recombinant Bacmide DNA. The recombinant Bacmide is transfected into Sf9 (*Spodoptera frugiperda*) insect cells for Baculovirus expressing recombinant GST-tagged ALK protein production (P1 stock).

- GST-3C r-ALK constructs:

The BacPACK Baculovirus expression system (Clontech) is used. The transfer vector (pBacPAK-GST-ALK KD) and the viral expression vector (digested BacPAK6) are cotransfected into Sf9 cells for recombinant Baculovirus production (P1 viral stock).

r-Baculovirus title increase

Virus title is increased from P1 to P3. 20 µL of the P1 are used to infect $2x10^6$ Sf9 cells for 5 days and the supernatant is collected (P2 viral stock). 20 µL of the P2 are used to infect $20x10^6$ Sf9 cells for 5 days and the supernatant is collected (pre-P3 viral stock). All the pre-P3 virus is used to infect $500x10^6$ Sf9 cells for 6 days and the supernatant is collected (P3 viral stock).

P3 r-Baculovirus title determination

$2x10^6$ Sf9 cells are infected considering different multiplicity of infection (MOI), 2 and 5, and different virus title for 3 days. The cells are collected and tested for r-protein expression by WB anti GST.

Expression of r-ALK protein in Sf9 insect cells

Sf9 cells are infected at a MOI of 5 or 2. Cells are cultured for 72 hours at 27ºC in suspension in Sf-900II medium (Invitrogen) supplemented with 80 µg/ml gentamycin, 100 U/ml penicillin, 2 mM glutamine and 1% Pluronic-F68 (Invitrogen). Infected Sf9 cells are harvested by centrifugation at 1500 rpm for 10 min at 4℃ and then stored at -80℃ until use.

In batch purification of r-ALK protein

$1*10^9$ frozen infected cells are lysed in 20 mL of Buffer A (50 mM Tris pH 8, 100 mM NaCl, 1 mM DTT, 0.1% Tryton x-100, 0.5 mM EDTA and PI) for 30 mins on ice. Cell lysates are ultracentrifugated at 16500 rpm for 30 minutes and the supernatant is incubate for 1 hour at 4℃ with glut athione-sepharose 4B beads (GE Healthcare) previously equilibrated in lyses buffer. For the phosphorylated form, 5 mM of ATP and 10 mM of $MgCl_2$ is added during the incubation. Beads are washed

3 times in Buffer A and 3 times in Buffer B (50 mM Tris pH 8, 100 mM NaCl, 1 mM DTT, and 0.5 mM EDTA) and incubated o/n with 50 µg of GST-3C protease in 5 mL of Buffer B. Cleaved protein is recovered.

In batch purification of r-ALK protein for STD-NMR studies

1*10^9 frozen infected cells are lysed in 20 mL of Buffer A (50 mM Tris pH 8, 100 mM NaCl, 1 mM DTT, 0.1% Tryton x-100, 0.5 mM EDTA and PI) for 30 mins on ice. Cell lysates are ultracentrifugated at 16500 rpm for 30 minutes and the supernatant is incubate for 1 hour at 4℃ with glut athione-sepharose 4B beads (GE Healthcare) previously equilibrated in lyses buffer. Beads are washed 3 times in Buffer A and 3 times in Buffer B (PBS buffer 1x pH 7 NaCl and 0.5 mM EDTA) and incubated o/n with 50 µg of GST-3C protease in 5 mL of Buffer B. Cleaved protein is recovered.

On column purification of r-ALK protein

1*10^9 frozen infected cells are lysed in 20 mL of Buffer A (100 mM Tris pH 8, 50 mM NaCl, 1 mM DTT, 10% glycerol and PI) for 30 mins on ice, ultracentrifugated at 16500 rpm for 30 minutes and the supernatant is filtrated through a 0.45 µm filter (Millipore, Bradford, MA, USA). Sample is loaded onto a GSTrap HP 4x5 mL column (GE Healthcare) previously equilibrated in Buffer A using the AKTA-FPLC system (GE Healthcare). GST-protein is eluted under an increasing concentration of Buffer B (100 mM Tris pH 8, 150 mM NaCl, 1 mM DTT, 10% glycerol and 20 mM of glutathione). Positive fractions are collected and incubated o/n 4℃ in 5 mL of TEV buffer (50 mM Tris pH8, 1 mM DTT, 0.5 mM EDTA and 1% glycerol) with TEV protein. Cleaved protein is recovered.

Production and purification of the Tobacco Etch Virus protease from bacteria

The Tobacco Etch Virus (TEV) protease is produced from BL21-pRK793 bacteria cultured in presence of ampicillin (100 µg/mL) and chloramphenicol (30 µg/mL). When the culture reaches an O.D.600 = 0.5-0.6, 1 mM IPTG is added. After 4 h at 30℃ cells are lysed in 50m M PO4 pH 8.0, 10 0 mM NaCl, 10% glycerol, 25 mM imidazole, 1 mg/ml lysozime and protease inhibitors. Cell lysate is sonicated (5 hits of 10 sec/low intensity) and 5% of polyethylenimine is added before ultracentrifuging for 30 minutes. Supernatant is loaded onto a NiNTA column equilibrated in BufferA (Lysis buffer without lysozyme) for affinity chromatography

using the AKTA-FPLC system (GE Healthcare). Proteins are eluted under an increasing gradient of imidazole with buffer B (50 mM PO4 pH8.0, 100 mM NaCl, 10% glycerol, 200 mM imidazole and protease inhibitors). Positive fractions of TEV protease are collected, concentrated and stored at -80℃ until use.

Western blot

Laemmli buffer (62.5 mM Tris-HCl, 2% SDS, 10% glycerol, 0.001% bromophenol blue, 5% ß-mercaptoethanol) is added to the Sf9 infected cells or to the r-ALK purified protein, sample is heated at 95°C for 10 min and centrifuged for 1 minute. Proteins are resolved using 10% SDS-PAGE gels and then transferred to an Immobilon TM-P membrane (Millipore) in the presence of transfer buffer (25 mM Tris-HCl, 192 mM glycine pH 8.3 and 20% methanol) at 80 V for 1 h. The membrane is blocked for 1 huor in 5% milk in wash I solution (20 mM Tris-HCl pH 7.4, 20 mM NaCl, 0.1% Tween-20). The membrane is incubated for 1 h at room temperature with primary antibody: monoclonal anti-GST antibody (Invitrogen), diluted 1:2000 in milk 2.5%, or monoclonal anti P-Y antibody (Millipore) diluted 1:1000 in BSA 2.5%. Membranes are washed in wash I buffer and incubated for 1 h with the secondary antibody: anti-rabbit horse-radish peroxidase (HRP) conjugated or anti-mouse HRP conjugated (Bio-Rad). Bands are visualized by ECL (Thermo Scientific) with Kodak.

Silver staining

Proteins are resolved by SDS-PAGE and the gel is then soaked in 7% acetic acid for 7 min and washed twice in 200 ml of 50% methanol for 20 minutes. The gel is rinsed two times in 200 mL water for 10 min each wash. The staining is performed by incubation with staining solution (0.8% silver nitrate, 1.4% 14 M ammonium hydroxide and 0.36% NaOH) for 15 min. The gel is rinsed again two times in 200 mL water for 5 min and finally developed in a solution containing 0.005% citric acid and 0.02% formaldehyde with shaking. The development is stopped by rinsing the gel 3 times with 200 mL water.

Coomassie staining

Proteins are resolved by SDS-PAGE and the gel is soaked in the comassie staining solution (10% acetic acid, 10% methanol and 0.5 g of comassie blue dye) for at least 1 hour. The gel is destained in the comassie destaining solution (10% acetic acid, 10% methanol).

Ponseau staining

Proteins are resolved by SDS-PAGE and transferred to the Immobilon TM-P membrane (Millipore). The membrane is soaked in the ponseau staining solution (0.1% Ponceau S in 5% acetic acid) for few minutes and later washed with the wash I solution.

Measurement of protein concentration using the Bradford assay

Protein concentration is determined using the Bradford assay. BioRad reagent is diluted 1:5 in water to a final volume of 1 mL and the sample is added. After incubation at room temperature for 5 min, the absorption is read at 595 nm using a spectrophotometer (Eppendorf). The concentration of protein in samples is determined using a standard curve generated with BSA at known concentrations (from 1 to 20 µg/mL).

Cold kinase assay

Purified protein is incubated with the reaction mix (1mM DTT, 25 mM Hepes pH7, 5mM $MgCl_2$ and 5 mM $MnCl_2$), with and without ATP 100 µM, in a final volume of 50 µL for 15 minutes at 30℃. The reaction is st opped adding Laemmli buffer and denaturating 10 minutes at 95℃.

Protein dialysis

10 µg (40 µL) of purified protein are dialyzed against Buffer B (50 mM Tris pH 8, 100 mM NaCl, 1 mM DTT, and 0.5 mM EDTA) for 6 hours using Millipore tubes.

r-ALK protein dephosphorylation

10 µg of purified protein are o/n incubated with 5 µL of phosphatase (Cip from New England Biolabs).

r-ALK protein concentration

Purified protein is centrifuged at 3000 rpm using centricon (vivaspin Sartorius stedim biotech), 10000 MW cut off. For crystallization trials, inhibitor is added 3 M in excess respect to the purified protein 10 minutes before concentration.

Size exclusion chromatography (SEC)

The Superdex 200 HR10/30 column (GE Healthcare) has been calibrated using Gel Filtration Molecular Weight Markers MW-GF-200 (Sigma) following manufacturers instructions. 100-200 µL of purified protein concentrated till 1 mg/mL are loaded onto the Superdex 200 HR 10/30 column. Isocratic elution is performed in buffer A at a flow rate of 0.4 mL/min. Buffer A contains 50 mM Tris HCl pH 8.0, 400 mM NaCl, 1 mM DTT and 0.5 mM EDTA. Using the standard curve obtained with the Molecular Weight Markers (retention vs. Log10MW) it is possible to determine the molecular weight of the protein.

Ion exchange chromatography (IEX)

An HiTrap Q HP 1 mL column (GE Healthcare) is used. The column is previously equilibrated in buffer A, purified protein is loaded under a flow rate of 1 mL/min and few column volume (CV) of buffer A are used for column wash. Protein is eluted under an increasing of buffer B concentration.

Buffer A used for ALK 6: 20 mM Tris pH 7, 30 mM NaCl, 1 mM DTT and 5% glycerol; buffer A used for ALK 7: 20 mM Tris pH 8.5, 30 mM NaCl, 1 mM DTT and 5% glycerol. Buffer B used for ALK 6: 20 mM Tris pH 7, 250 mM NaCl, 1 mM DTT and 5% glycerol; buffer B used for ALK 7: 20 mM Tris pH 8.5, 250 mM NaCl, 1 mM DTT and 5% glycerol.

Enzyme-linked immunosorbent assay (ELISA)

Immuno plates (Nunc) are coated with peptide substrate (ARDIYRASFFRKGGCAMLPVK) at 2.5 µg/well in 125 µl PBS by incubation o/n at 37℃. Wells are washed with 200 µL of wash buffer (PBS-Tween 0.05 %) and dried for 2 h at 37℃. The kinase reaction are performed by incubating kinase buffer (50 mM Tris pH 7.5, 5 mM $MnCl_2$, 5 mM $MgCl_2$), 0.3 mM ATP and purified r-ALK (50 ng/well) in a total volume of 100 µl/well at 30℃ for 10 min. When testing inhibitors, the reaction mix is pre-incubated with the inhibitor or solvent control for 10 min at room temperature in a standard 96-well plate before transferring to the ELISA plate. Phosphorylated peptide is detected using a monoclonal anti-phosphotyrosine antibody (Millipore) diluted 1:2000 in PBS and 4% BSA (100 µl/well). After 30 min of room temperature incubation, wells are washed 5 times and a secondary antibody (anti-mouse IgG-HRP linked antibody, BioRad) diluted 1:1000 in PBS and 4% BSA

is added. The plate is incubated and washed as before, then developed using 100 µl/well TMB (tetramethylbenzidine) Substrate Solution (Endogen) and 0.18 M H2SO4 stop solution. The absorbance is read at 450 nm using an Model 680 microplate reader (Bio-Rad). EC50 values are determined by GraphPad Prism software fitting the data using nonlinear regression.

Thermal denaturing assay

The thermal shift assay is performed in the Mx3005P Realtime PCR (Sratagene) originally designed for real time PCR. Solutions of 5 µl of 1 mg/mL of purified r-ALK, 5 µL of Sypro Orange (diluted 100x in H_2O) (Invitrogen) and 90 µL of buffer (refer to results) are added to the wells of the 96-well PCR plate. The plate is heated from 25 to 89℃ at a rate of 1℃/min. The fl uorescence intensity is measured.

Crystallization trials

The vapour diffusion method has been used for crystallization attempts. Different crystallization kits have been used: $AmSO_4$ Suite, Classic Lite Suite, PEGs Suite, MPD Suite, Ph Clear Suite, Ph Clear II Suite, PACT Suite, JCSG Core Suite I, JCSG Core Suite II, JCSG Core Suite III, JCSG Core Suite IV (Qiagen), HR2-086, HR2-098, HR2-130, HR2-133, HR2-134, HR2-136, HR2-137, HR2-139 (Hampton Research), Clear Strategy Screen I, Clear Strategy Screen II, Structure Screen 1&2 (Molecular Dimensions) (**Appendix 1**). 100 µL of different tested precipitated solution are dispensed in each reservoir of the 96-well sitting drop iQ plate (TTP LabTech). 100, 150 or 200 nL of purified concentrated r-ALK protein are seeded and mixed together with the precipitated solution at 1:1 or 1:2 (solution:protein) ratio using Mosquito Crystal (TTP LabTech). Plates are stored at 20 or 4℃.

STD-MNR

All of the experiments are recorded on a Varian 400 MHz instrument. The ligand resonances are assigned by using ^1H–^1H COSY, ^1H-^1H TOCSY, ^1H-^1H NOESY and ^1H–^{13}C HSQC NMR spectroscopy. A basic 1D-STD sequence is used with the on-resonance frequency of -1 ppm and the off-resonance frequency of 40 ppm. A train of Gaussian-shaped pulses of 50 ms each is employed, with a total saturation time of the protein envelope of 4s. The total saturation time is adjusted by the number of shaped pulses. A T11 filter of 2 ms is employed to eliminate the

background signals from the protein. All of the samples are dissolved in deuterated PBS + 10% DMSO, at 30℃. Compound R458 is tested wi th a final concentration of 1mM, whereas ALK concentration is ≈ 8 μM. Total sample volumes are 550 mL. The on- and off-resonance spectra are acquired simultaneously with the same number of scans (1024 total scans). The STD spectrum is obtained by subtraction of the on-resonance spectrum from the off-resonance spectrum. Subtraction is performed by phase cycling to minimize artifacts arising from magnet and temperature instabilities (Mayer, Meyer et al. 2011). Reference experiments of samples containing only the free compounds tested are performed under the same experimental conditions to verify true ligand binding. The effects observed in the presence of the protein were due to true saturation transfer, as no signal was present in the STD spectra obtained in the reference experiments, except residues from HDO, indicating artifacts from the subtraction of compound signals to be negligible. Spectra processing and analysis were performed with the software MestReNova (http://mestrelab.com/).

RESULTS

GST-TEV r-ALK CONSTRUCTS

PRODUCTION OF r-ALK CONSTRUCTS

We used the Bac-to-Bac Expression System, as the first method, in order to obtain r-ALK protein. In particular, the cytoplasmic portion of the protein, including the kinase domain, was cloned into the pDEST20 expression vector. The recombinant Bacmid was then transfected into Sf9 (*Spodoptera frugiperda*) cells for recombinant Baculovirus production and the virus obtained was used for insect cells infection (**Fig 11**). pDEST20 vector is characterized by a Glutathione S-transferase (GST) tag sequence at the N-terminal portion, that facilitates protein purification by affinity chromatography. The GST-tag is also very useful to increase recombinant protein stability and solubility and it leads to kinase domain dimerization as the partner protein NPM in the oncogenic fusion protein NPM/ALK. In addition, the vector includes a Tobacco etch virus (TEV) protease recognition site to remove the GST-tag after the purification in order to reduce problems in structural determination. TEV is a cysteine protease that recognizes the cleavage site of Glu-Asn-Leu-Tyr-Phe-Gln-Gly and cleaves between Gln and Gly.

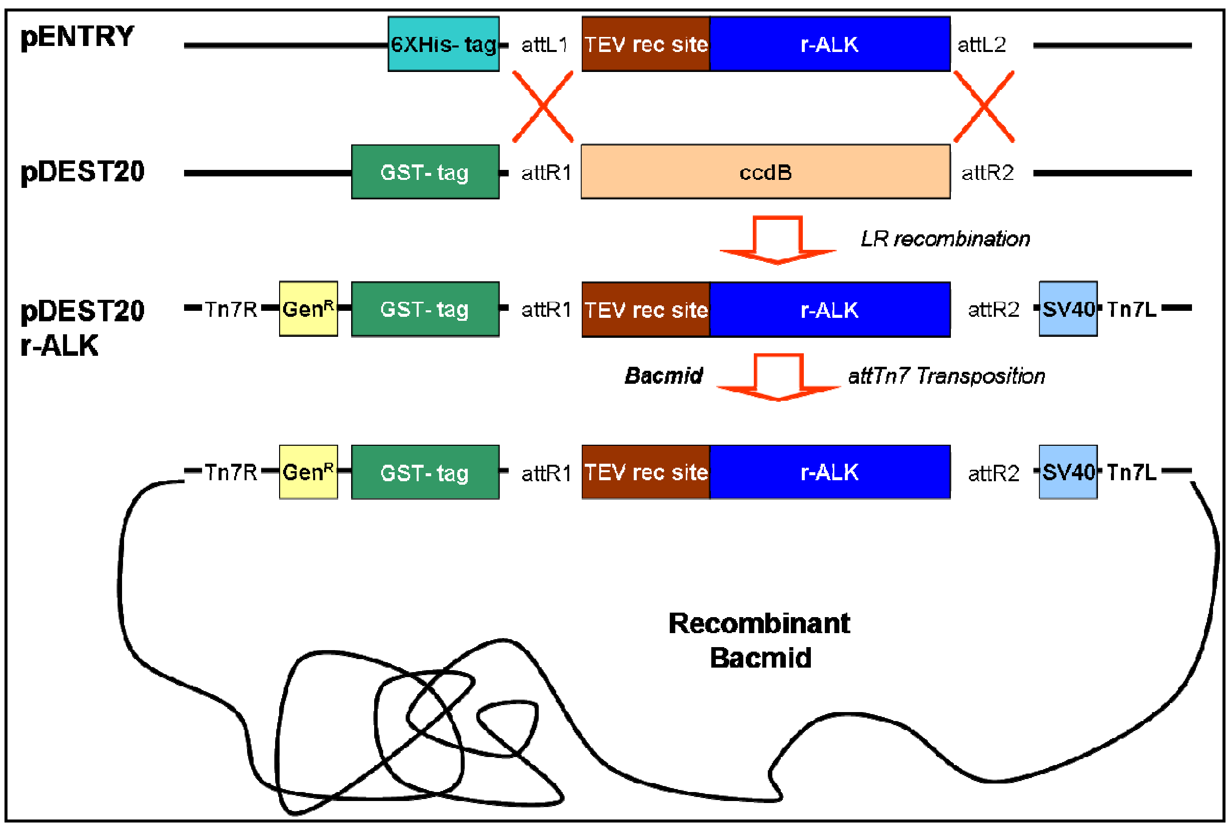


Fig 11_ Production of r-ALK according to the Bac-to-Bac Expression System. *After LR recombination between the pENTRY containing the r-ALK sequence and the pDEST20, containing the GST-tag sequence, the GST-ALK sequence is inserted into the Bacmide by Sf9 cells transfection for r-Baculovirus production.*

Four ALK sequences have been cloned into pDEST20 to obtain a useful protein for ALK structural study. All these sequences (called ALK1, ALK2, ALK3 and ALK4) include the kinase domain starting from the residue 1099 of ALK full length and ending at residues 1397, 1410, 1435 and 1620 respectively (**Fig 12**). In particular, ALK 2 and 3 have been chosen because of similarity to the IRK already crystallized protein, this gives more chances for their own crystallization. As seen in the following alignment, there are correspondences both for the DFG motif and the YxxxYY motif between ALK constructs and the IR (**Fig 13, 14**). The ALK full length have instead been considered to study the importance of the C-terminal tail of the kinase in protein stability and regulation.

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGGSRIAYSKDFE
TLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFP
KLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGLQATFGGGDHPPKSDRVPRHNQTSLYK
KAGSAAAVLEENLYFQ/GSFTAGKTSSISDLKEVPRKNITLIRGLGHGAFGEVYEGQV
SGMPNDPSPLQVAVKTLPEVCSEQDELDFLMEALIISKFNHQNIVRCIGVSLQSLPRF
ILLELMAGGDLKSFLRETRPRPSQPSSLAMLDLLHVARDIACGCQYLEENHFIHRDIA
ARNCLLTCPGPGRVAKIGDFGMARDIYRASYYRKGGCAMLPVKWMPPEAFMEGIFTSK
TDTWSFGVLLWEIFSLGYMPYPSKSNQEVLEFVTSGGRMDPPKNCPGPVYRIMTQCWQ
HQPEDRPNFAIILERIEYCTQDPDVINTALPIEYGPLVEEEEKVPVRPKDPEGVPPLL
VSQQAKREEERSPAAPPPLPTTSSGKAAKKPTAAEVSVRVPRGPAVEGGHVNMAFSQS
NPPSELHKVHGSRNKPTSLWNPTYGSWFTEKPTKKNNPIAKKEPHDRGNLGLEGSCTV
PPNVATGRLPGASLLLEPSSLTANMKEVPLFRLRHFPCGNVNYGYQQQGLPLEAATAP
GAGHYEDTILKSKNSMNQPGP

Fig 12_ r-ALK amino acid sequences cloned into pDEST20 vector.

In green is the GST-tag sequence, in brown the TEV protease recognition size, in red the ALK kinase domain and in blue is the ALK sequence; the slash indicates the cleavage site. The ALK 1, 2, 3 and 4 construct ends are shown in yellow, light green, violet and black respectively.

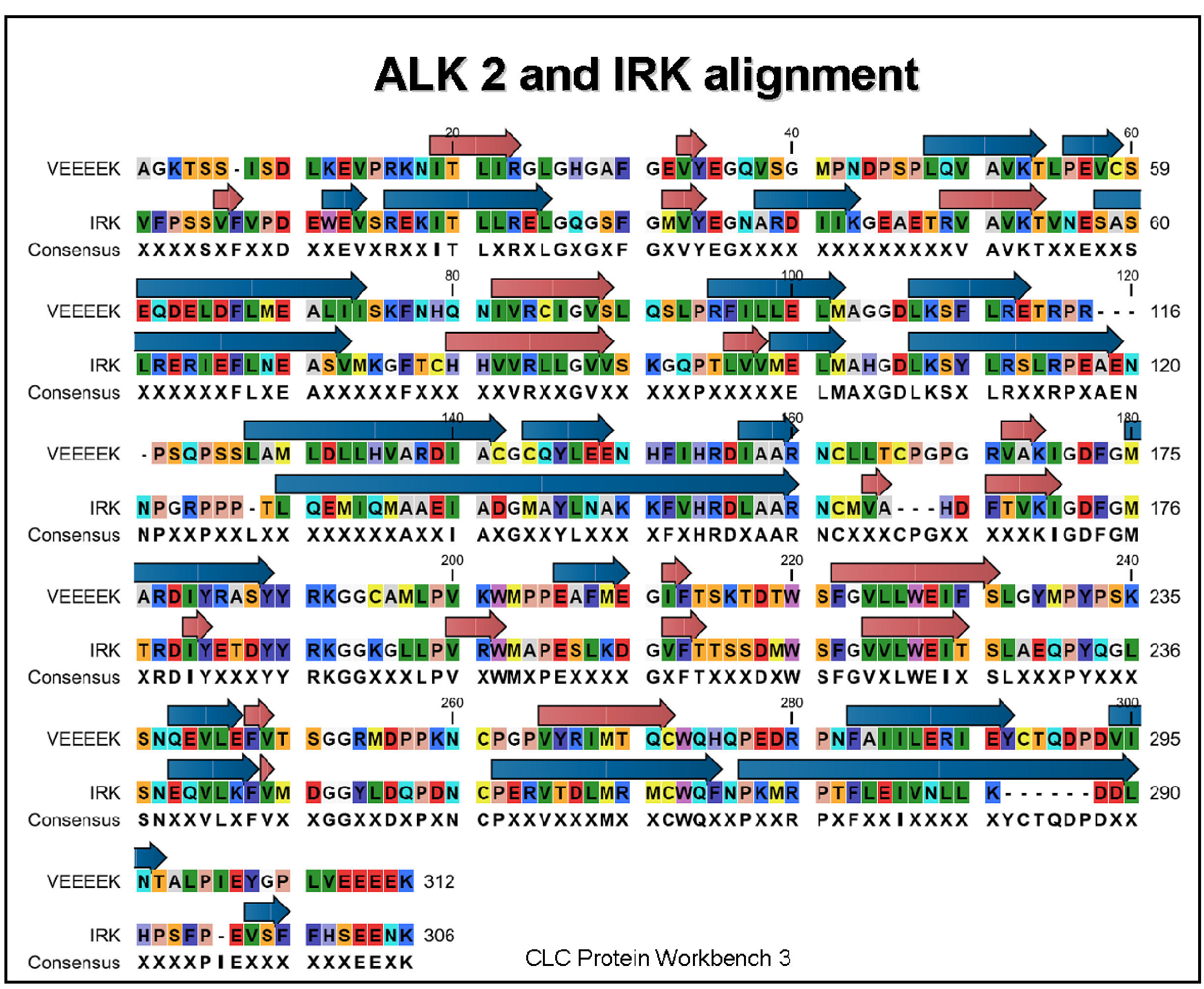


Fig 13_ Alignment between the ALK 2 sequences and the IRK.

The ALK 2 sequence (indicated as VEEEEK protein) is in the first line, the IRK is in the second and the consensus is shown in the third line.

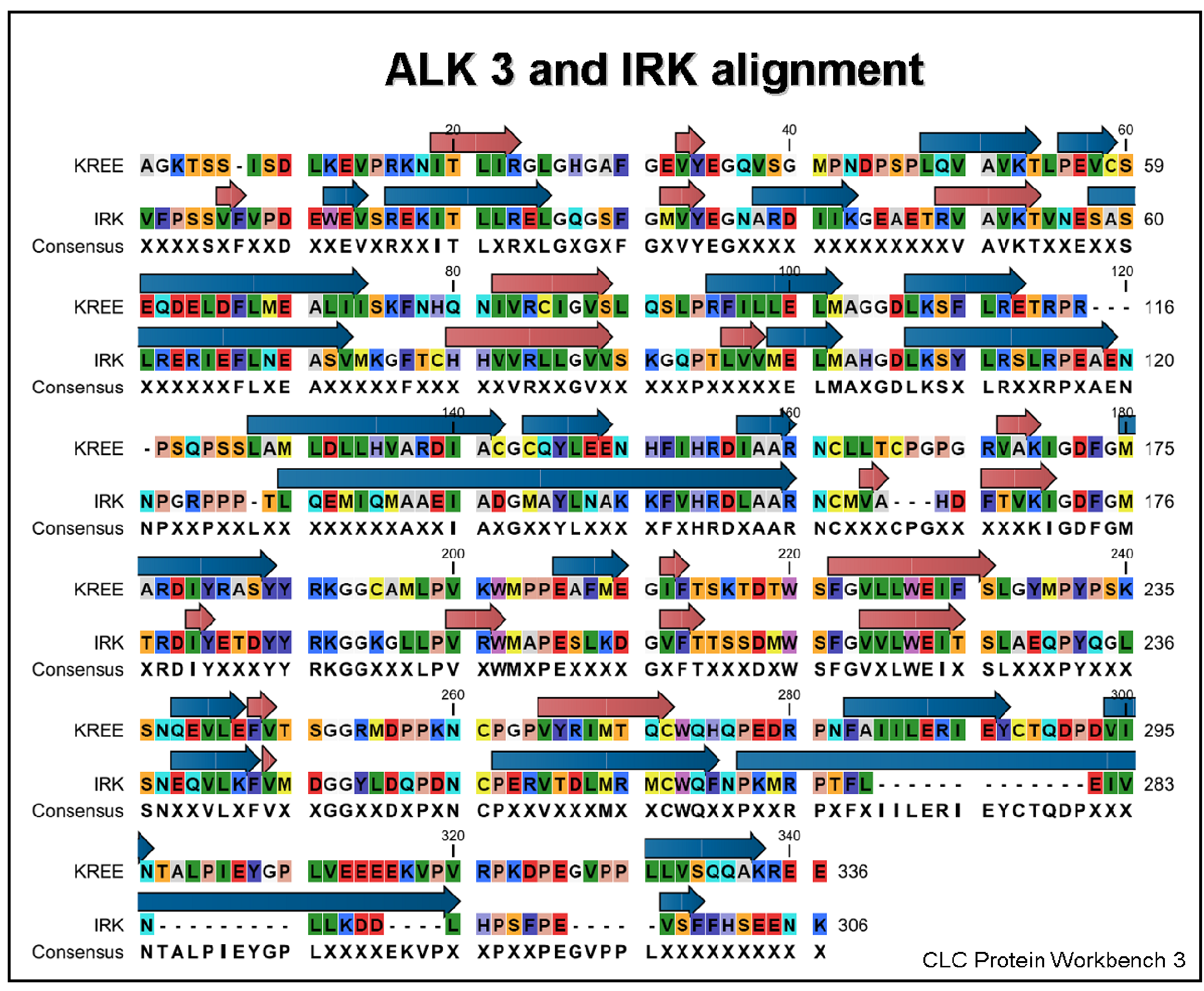


Fig 14_ Alignment between the ALK 3 sequences and the IRK.

The ALK 2 sequence (indicated as KREE protein) is in the first line, the IRK is in the second and the consensus is shown in the third line.

OPTIMIZATION OF r-ALK PURIFICATION PROCEDURES

After the constructs production, we focused our attention on the optimization of procedures for the different protein purification steps. In particular, we considered:

- the lysis condition;
- the GST-tag cleavage process;
- the purification protocol (in batch or on column purification).

OPTIMIZATION OF LYSIS CONDITION

The use of an optimized buffer for r-Baculovirus infected Sf9 cells lysis is important to improve the amount of recovered soluble protein. GST-ALK 1 protein has been used as model for the other GST-ALK recombinant proteins, the procedure of lysis optimized for this protein has been considered also for the others.

Different lysis conditions have been tested, all including protease inhibitors (Aprotinin 0.5 µg/mL, Leupeptin 0.5 µg/mL, Pepstatin 1 µg/mL, Benzamydin 1 Mm). We changed pH (7.4 or 8), NaCl concentration (from 20 to 150 mM), buffer composition using Tris or Hepes and we also explored the effect of the detergent tritonX-100 and glycerol addition. The same number of infected cells have been lysed using different types of lysis buffer, the sample has been incubated for 15 minutes on ice and centrifuged 4000 rpm for 30 minutes. Each buffer efficiency has been analyzed by Wester Blot (WB) using the anti-GST antibody, both in the soluble fraction (surnatant, S) and in the insoluble one (pellet, P) (**Fig 15).** We can observed that the condition number 13 (100 mM Tris pH 8, 50 mM NaCl, 10% glycerol, PI) is the best condition tested because of the presence of a bigger amount of protein (GST-ALK) in the soluble portion.

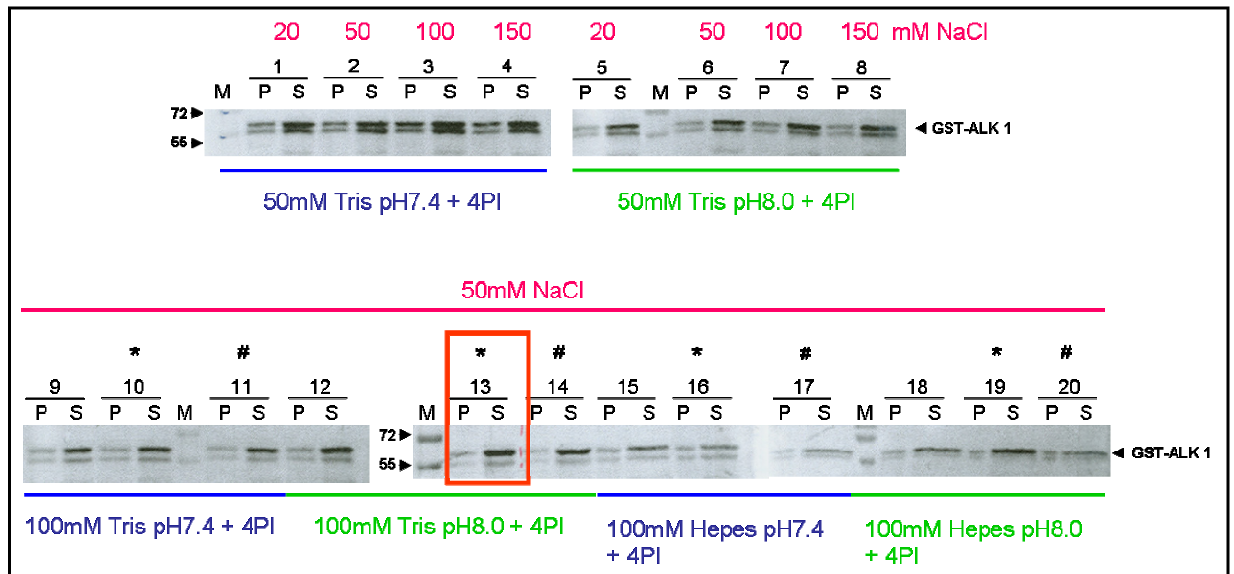


Fig. 15_ Optimization of lysis condition.

*WB anti-GST of Sf9 infected cells lysed with different buffers. 20 different conditions have been tested (from 1 to 20), considering pellet (P) and supernatant (S) from each condition. The best condition is marked in red. M = molecular weight marker, * = addition of 10% glycerol, # = addition of 1% triton X-100, PI = protease inhibitors.*

OPTIMIZATION OF THE GST-TAG CLEAVAGE

Another important step to obtain an high quality of purified protein is the complete tag removal. Therefore, optimization of the GST-tag cleavage mediated by TEV protease is necessary.

2 x 10^9 of Sf9 infected cells have been lysed with the optimized lysis buffer and the GST-ALK 1 protein has been purified according to the on column purification (see below). The GST-ALK 1 purified protein (2,8 mg) has been used to test different cleavage conditions verified by Coomassie Brilliant Blue staining. Half of the sample has been concentrated 3 times (3x) adding TEV protease 1:50 (TEV:protein ratio based on concentration) and TEV buffer (50mM Tris pH 8, 1mM DTT, 500 µM EDTA) 1x, 5x or 10x. The other half of the protein has not been concentrated and different TEV:protein ratios (1:5, 1:10, 1:30, 1:50, 1:100) have been considered. It is evident that there is no difference between the concentrated GST-ALK control (lane 2) and the non-concentrated one (lane 5) in terms of ALK amount (**Fig 16**). Therefore, concentration should not be performed before cleavage, because it can cause protein loss. Moreover, ALK is not stable in presence of TEV buffer 1x, this is demonstrated by the appearance of a white pellet (lanes 8 and 9) and by the reduction of recovered ALK protein. The same is in

presence of TEV buffer 5x (lane 13). This is probably due to the dilution of salts in protein buffer caused by the TEV buffer 1x or 5x addition. Our results suggested that salts contribute to protein stability, in fact buffer 10x addition did not produce precipitation. Regarding TEV:protein ratio, 1:50 is sufficient to obtain a complete cleavage, in fact no more GST-ALK protein is present; while using 1:100 ratio, the band corresponding to ALK is lighter, suggesting the presence of a non-cut protein.

In conclusion, the optimized GST-tag cleavage conditions include a TEV:protein ratio of 1:50 in terms of concentration, TEV buffer 10x or no buffer addition in presence of a non-concentrated sample (**Fig 16**).

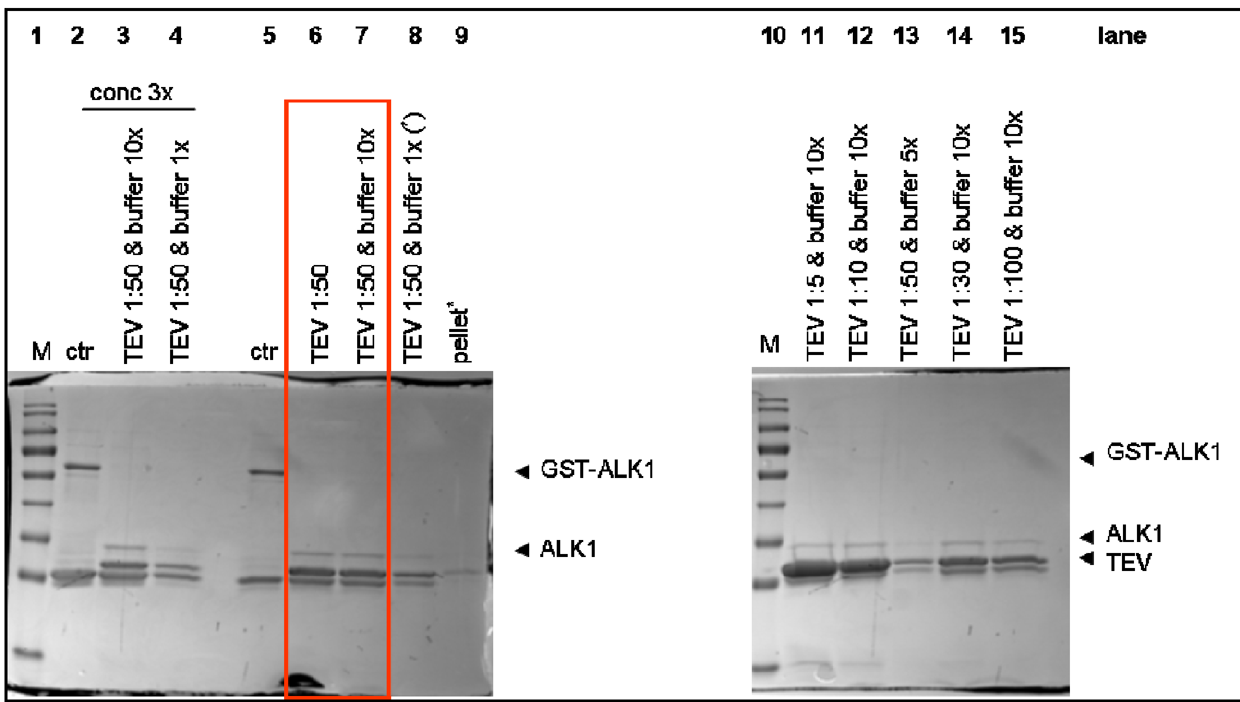


Fig 16_ Optimization of the GST-tag cleavage.

Coomassie Brilliant Blue staining of different conditions tested for GST-tag cleavage optimization. The protein is concentrated 3 times in the first three lanes after the M, it is non-concentrated in the other lanes. Different TEV:protein ratios and different TEV buffers have been used. Pellet is derived from the signed condition (*). The best cleavage condition is marked in red. M = molecular weight marker, ctr = non-cleaved GST-ALK 1.

“IN BATCH” OR “ON COLUMN” PURIFICATION

We analyzed two methods for GST-ALK proteins purification, in batch and with the AKTA FPLC system purification (**Fig 17**) using ALK 1 recombinant protein, to find out the best purification procedure in terms of sample purity and amount.

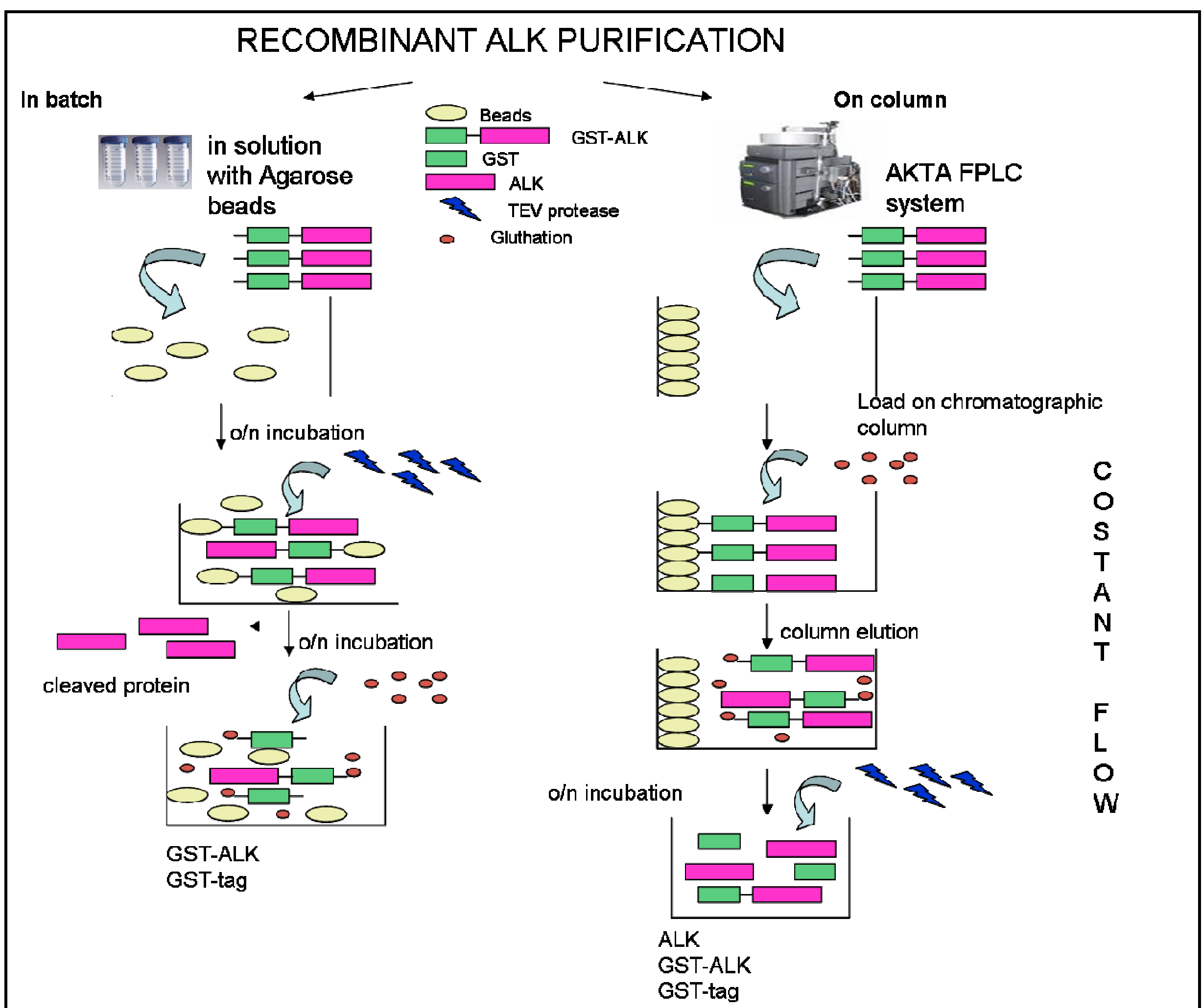


Fig 17_ Scheme of purification procedures in batch and on column with the AKTA FPLC System.

In batch purification

The clarified lysed sample was incubated overnight (o/n) with GST-Agarose beads for binding, then unbound protein was removed and TEV protease was added for o/n cleavage. In this case the final unbound fraction of sample should include the cut ALK protein; while the uncut one and the GST-tag should remain bound to the beads (**Fig 17** on the left). The WB analysis displays how, in the in batch purification, a lot of GST-ALK was lost in the unbound fraction and some was again lost during washes after binding (W1) (**Fig 18**). There was still un-cut protein bound to the resin after cleavage (in cut and elutions fractions) (**Fig 18** on the right). In

conclusion, in batch purification for GST-TEV r-ALK protein produced a big loss of protein, not able to bind the beads. The TEV cleavage was not complete and moreover there was TEV protease and GST-ALK in the cleaved fraction together with ALK (**Fig 18** on le left). This made further steps necessary to improve sample purity.

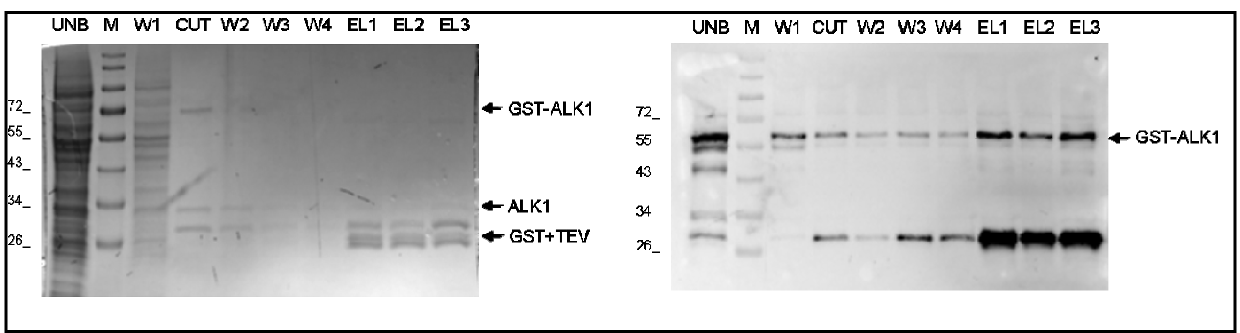


Fig 18_ ALK 1 in batch purification.

Coomassie Brialliant Blue (on the left) and Western Blot anti-GST (on the right) of in batch ALK 1 purification. UNB = unbound protein of the GST-beads; M = molecular weight marker; W1 = beads wash after protein binding; CUT = cleaved protein recovered; W2,W3,W4 = beads washes after protein cleavage; EL1,EL2,EL3 = protein elutions from the beads after cleavage.

After in batch purification, ALK and TEV protease are co-purified and they are both present in the cut fraction. We performed both size exclusion (SEC) and ionic exchange (IEX) chromatography to separate ALK, but the proteins were always eluted together probably because of their very closed molecular weight. Therefore, our results demonstrated that chromatography does not allow GST-TEV r-ALK protein isolation.

On column purification

In order to perform the on column purification, we used affinity chromatography (AC) (Akta FPLC System). The clarified lysed sample was loaded on a GST trap HP 4x5 mL column using the optimized buffers described in the methods. To increase protein-beads binding and the following elution, we used a low speed flow (0.5 mL/min). The eluted fractions, containing the GST-ALK protein, have been pooled and cleaved o/n adding TEV protease (**Fig 17** on the right). In this case, fractions under the chromatographic peak contain the recombinant protein and a contaminant (GST) (**Fig 19 A**). Positive fractions have been separately cleaved with success (**Fig 19 B**).

Analysing the on column purification (**Fig 19**), in comparison with the in batch purification, we have observed:

1. an higher amount of purified and recovered protein;
2. a complete GST-tag cleavage.

Considering these advantages given by on column purification, we decided to adopt this method for all the GST-TEV r-ALK proteins produced in order to improve the quality of purified protein achieving requested criteria for crystallization trails.

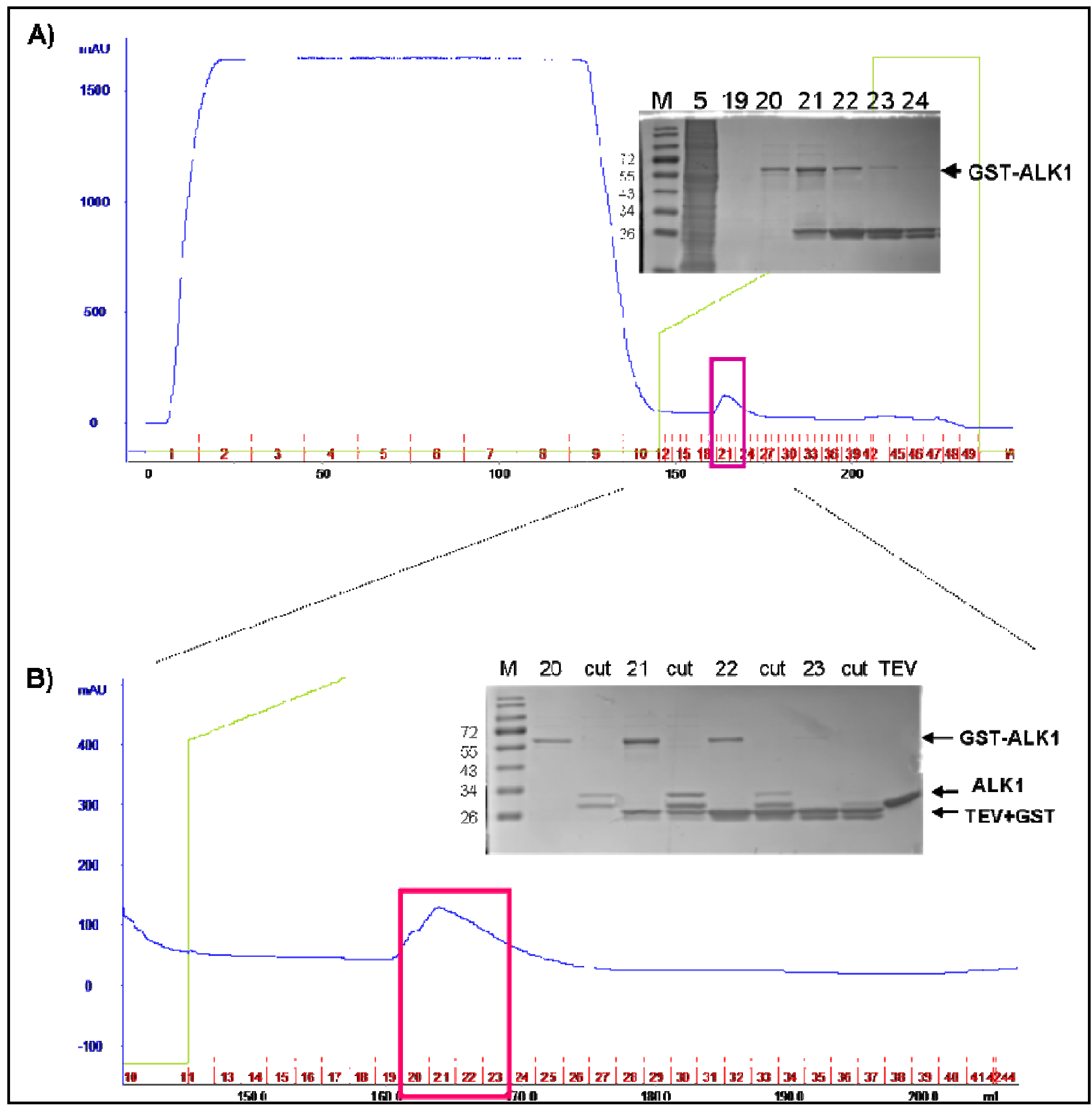


Fig 19_ ALK 1 on column purification and cleavage.

A) chromatogram resulted from AC and Coomassie Brilliant Blue analysis of the fractions (5 = unbound; 19/24 = fractions under the peak). B) zoom of the chromatographic peak and Coomassie Brilliant Blue of the protein cleavage, each fraction is shown before (numbered) and after the cleavage (cut). In the last lane is the TEV protease as control.

ALK 1 PROTEIN ISOLATION

We first produced the ALK 1 recombinant protein (A1099-L1397) characterized by a GST-TEV tag (**Fig 12**).

For this construct, we obtained r-ALK1 together with contaminations (TEV and GST) in positive fractions eluted after affinity chromatography as shown in the Coomassie gel (**Fig 19**) of a representative purification. In order to isolate the ALK 1 protein, we concentrated the cleaved protein up to 20 times before performing a size exclusion chromatography, a method which is able to separate proteins according to their size. Unfortunately, SEC did not give the expected result, in fact the chromatographic peak includes again ALK 1, TEV protease and GST-tag (**Fig 20**). This result suggested that the too similar proteins molecular weight caused the SEC failure in separating ALK 1.

We further used IEX, which is able to separate proteins according to their charge, to obtain pure ALK 1. Anyway, the result was similar to the SEC one.

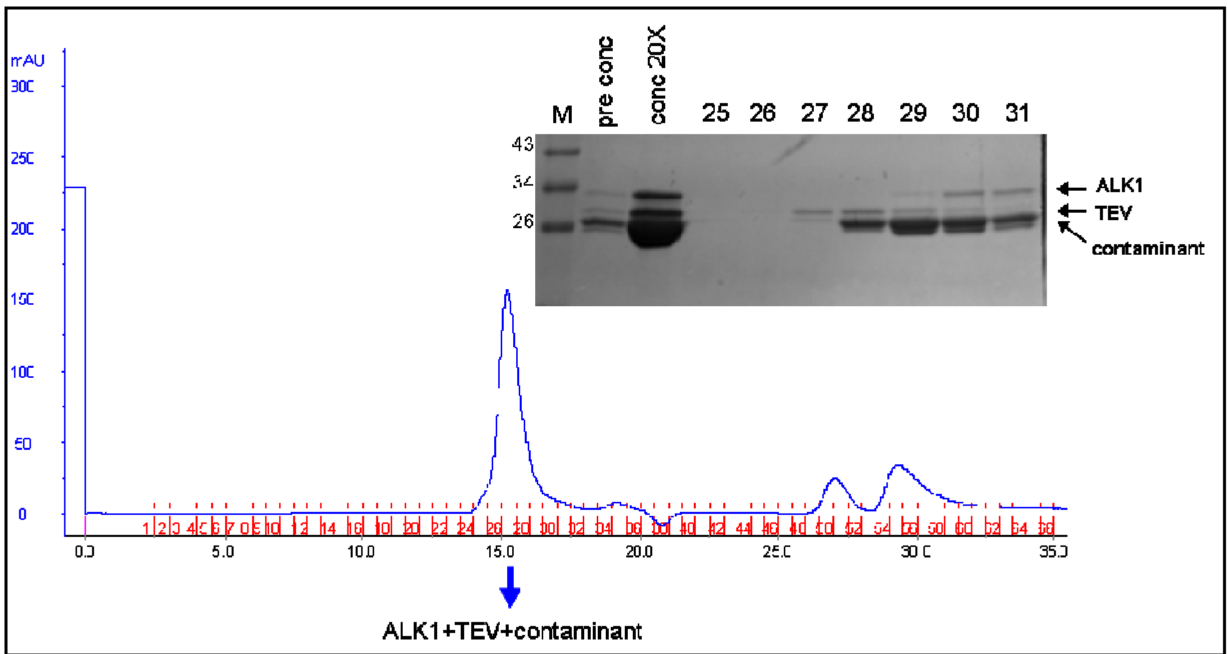


Fig 20_ SEC of ALK 1 cleaved protein.

Chromatogram resulted by SEC of ALK 1 cleaved protein and corresponding Coomassie Brilliant Blue. Pre conc = cleaved protein pool; conc 20x = cleaved protein pool concentrated 20 times before loading on column; 25-31 = fractions under the chromatographic peak.

During the optimization of protein purification trials we characterized this construct and, in order to assess the ALK 1 activity, we performed a cold kinase

assay. The WB revealed cleaved protein activity, as demonstrated by auto-phosphorylation, in presence of ATP (**Fig 21**).

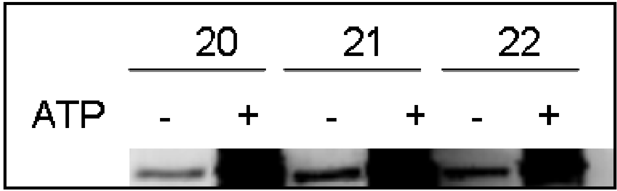


Fig 21_ ALK 1 cold kinase assay.

WB anti phosphotyrosine of cleaved fractions non-treated (-) or treated (+) with ATP.

In this case, after optimization of purification methods, we were able to obtain an active ALK 1 protein, but without contaminant completed removal. Moreover, during following experiments, some protein precipitated during cleavage and concentration probably because of the construct instability.

ALK 2 PURIFICATION

GST-ALK 2 (A1099-K1410) (**Fig 12**), characterized by the GST-TEV tag, has been purified according to the previously optimized conditions. The Coomassie analysis after the AC and GST-ALK 2 cleavage, revealed a good level of protein recovery and an almost completed GST-tag removal in each positive fraction (**Fig 22**).

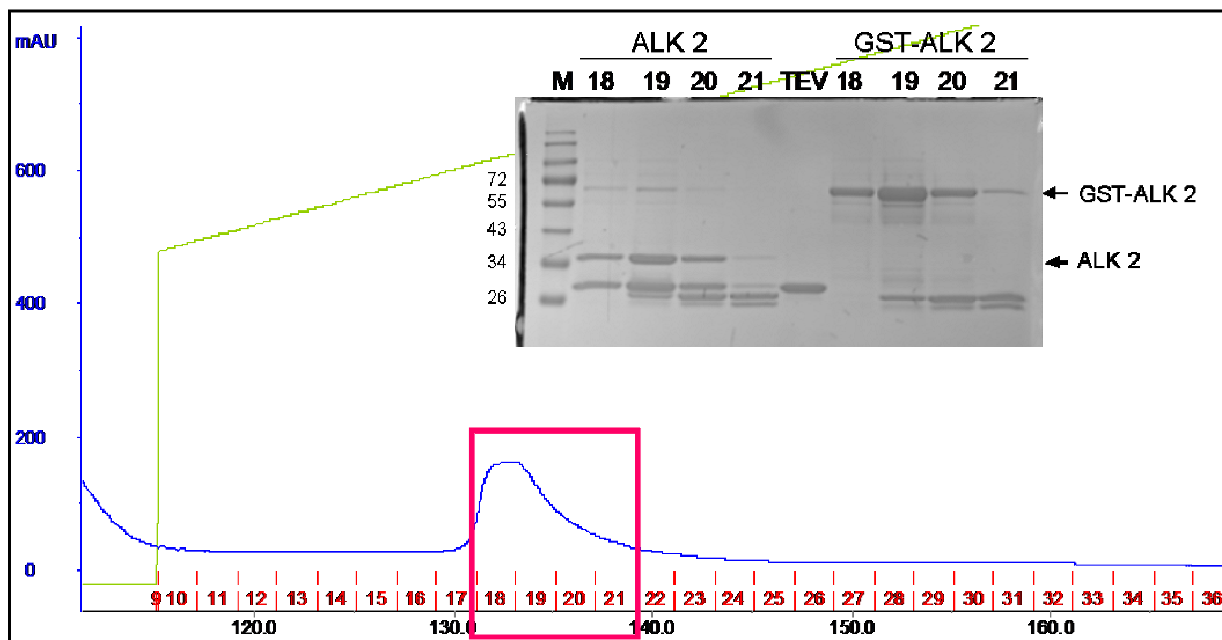


Fig 22_ ALK 2 on column purification and cleavage.

Zoom of the chromatographic peak and Coomassie Brilliant Blue showing fractions before (GST-ALK 2 on the right) and after the cleavage (ALK 2 on the left).

After cleavage process we tried to separate ALK 2 from GST and TEV protease by SEC. Unfortunately, the peak of eluted sample includes also contaminant together with the recombinant protein (**Fig 23**).

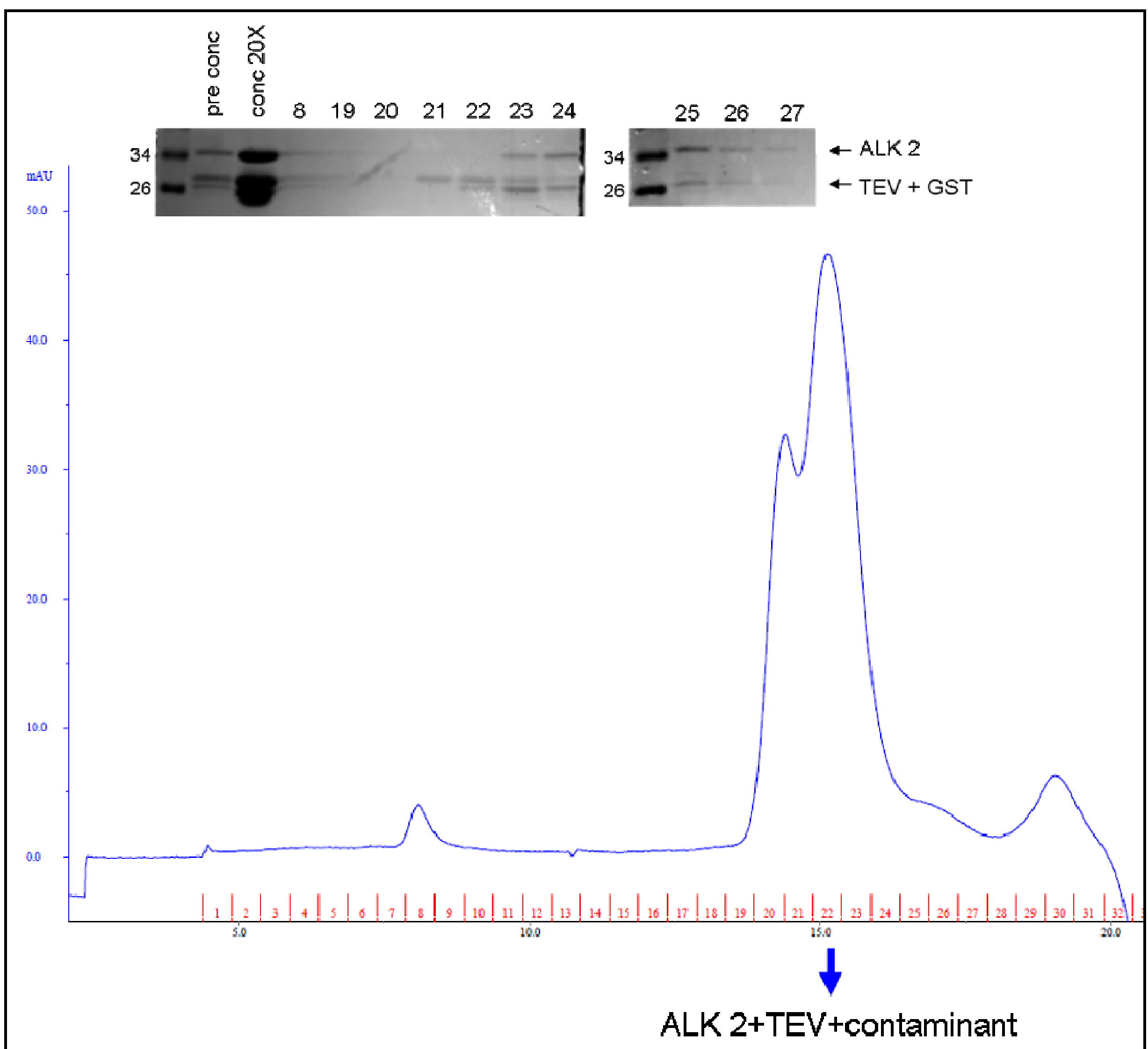


Fig 23_ SEC of ALK 2 cleaved protein.

Chromatogram resulted from SEC of ALK 2 cleaved protein and corresponding Coomassie Brilliant Blue. Pre conc = cleaved protein pool; conc 20x = cleaved protein pool concentrated up to 20 times before loading on column; 8,19-27 = fractions corresponding to the SEC peaks.

Even if the cold kinase assay revealed the ALK 2 activity (**Fig 24**), the ALK 2 protein did not display enough stability, precipitating after tag cleavage and also during the concentration process.

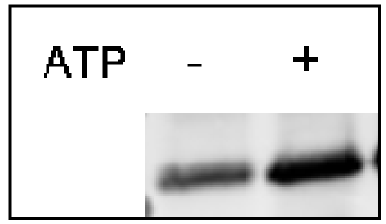


Fig 24_ ALK 2 cold kinase assay.

WB anti phosphotyrosine of cleaved fraction non-treated (-) or treated (+) with ATP.

For that reasons we produced a different recombinant ALK construct.

ALK 3 PURIFICATION

The third construct purified according to the optimized conditions was GST-ALK 3 (A1099-E1435), characterized by the GST-TEV tag (**Fig 12**). In this case, the TEV protease did not work with the purified protein (**Fig 25**), probably because of GST-ALK 3 conformation, which did not allow the protease binding.

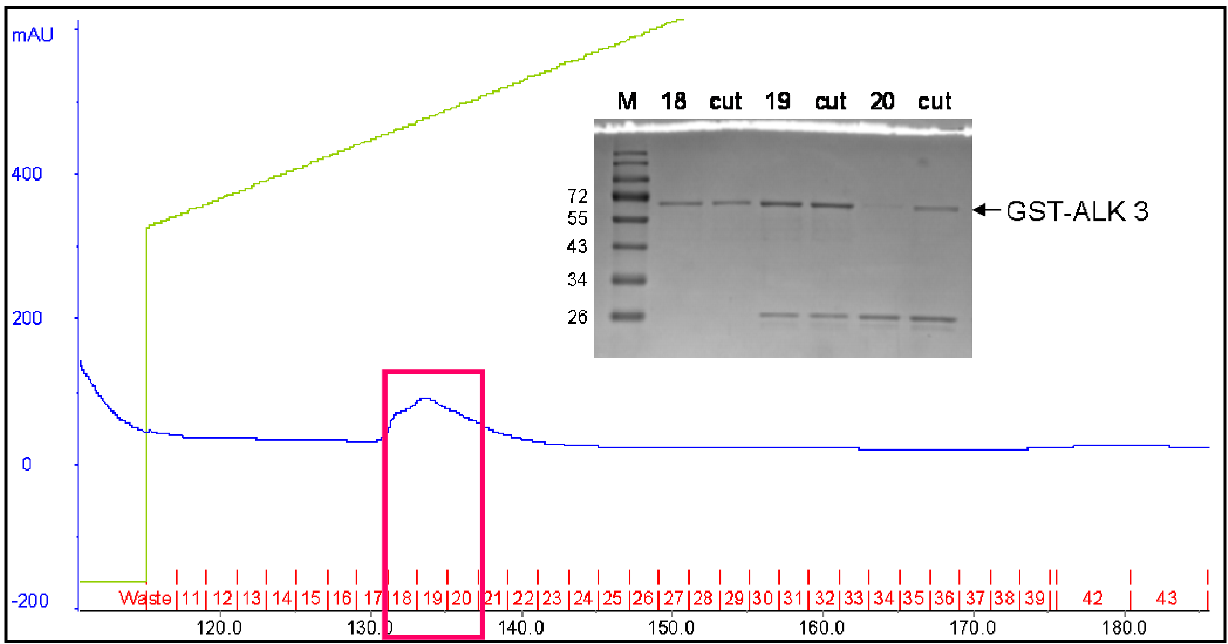


Fig 25_ ALK 3 on column purification and cleavage.

Zoom of the chromatographic peak and Coomassie Brilliant Blue, each fraction eluted under the peak is shown before (numbered) and after the protease addition (cut).

Also in this case, we eluted an active purified protein, as demonstrated in the cold kinase assay (**Fig 26**).

Fig 26_ ALK 3 cold kinase assay.

WB anti phosphotyrosine of cleaved fraction non-treated (-) or treated (+) with ATP.

Unfortunately, even if many efforts have been done to improve the purity of the final purified ALK3 protein we did not obtain positive results, therefore, we considered generation of another construct.

ALK 4 PURIFICATION

The ALK 4 protein (A1099-P1620) is characterized by the GST-TEV tag (**Fig 12**). As the other three proteins, also GST-ALK 4 has been purified by the on column method and cleaved in batch. In a representative experiment we observed that, in this case we obtained purified protein (**Fig 27**).

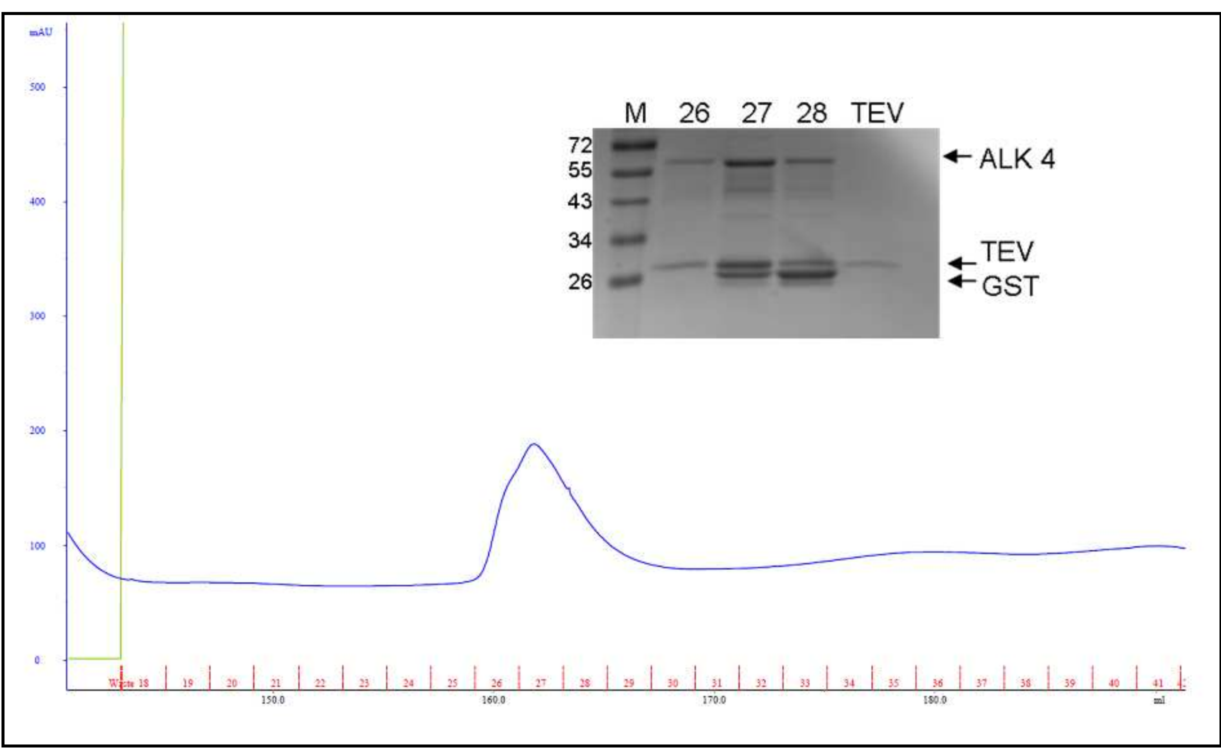


Fig 27_ ALK 4 on column purification and cleavage.

Zoom of the chromatographic peak of AC and Coomassie Brilliant Blue of the cleaved protein. Positive fractions for GST-ALK 4 (26,27,28) have been separately cleaved, in the last lane is the TEV protease as control.

In order to eliminate low molecular weight contaminants, we used SEC. In this case, chromatography was able to separate ALK 4 thanks to the different molecular weights of the involved proteins (**Fig 28)**. In the end we obtained the ALK 4 at higher level of purity than that of previously analyzed proteins because of the SEC efficiency. Anyway, we recovered low amount of purified protein, not sufficient for crystallographic trials.

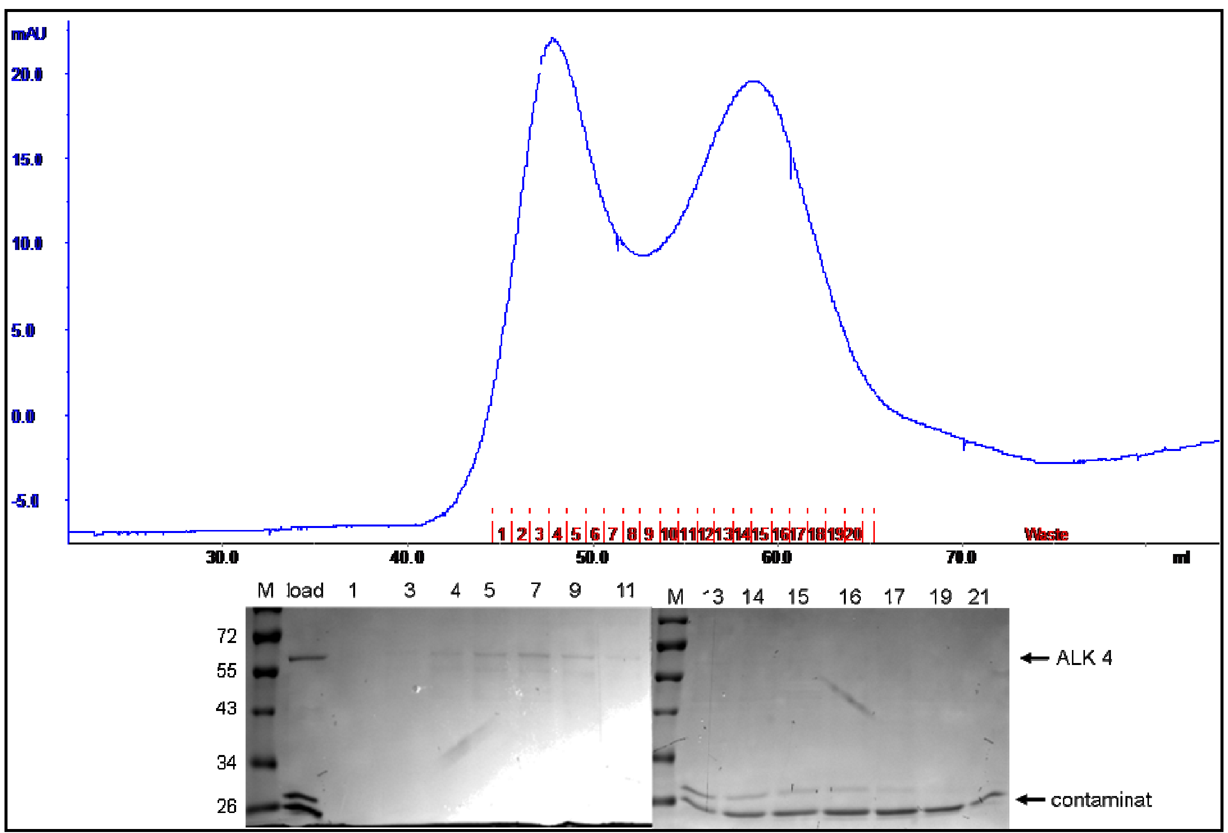


Fig 28_ SEC of ALK 4 cleaved protein.

Chromatogram resulted from SEC of ALK 4 cleaved protein and corresponding Coomassie Brilliant Blue. Load = sample loaded on column; from 1 to 21 = fractions corresponding to the peaks.

The four r-ALK constructs considered till now have been expressed according to the Bac-to-Bac Expression System. Looking at the proteins purification results, we can conclude that this system is not enough efficient for the production of a protein suitable for structural analysis by STD-NMR or X-ray crystallography. In particular, proteins are expressed at very low level and the presence of low molecular weight contaminants (GST and TEV protease) in the final product represents a serious obstacle to achieve requested level of purity.

To improve r-protein production, we decided at this point to consider a different expression vector: the BacPAK vector for production in Sf9 cells system.

GST-3C r-ALK CONSTRUCTS

PRODUCTION OF r-ALK CONSTRUCTS

The string of DNA coding for ALK kinase domain has been cloned into the pBacPAK vector, a pBacPAK-His3 vector modified by Prof Neil McDonald's Laboratory, for expression in Sf9 cells (**Fig 29**). The novel pBacPAK expression vector displays a GST-tag sequence at the N-terminal, as the pDEST20, and the recognition site for the GST-3C protease, a different protease respect to previous considered constructs. The advantage of the modified vector is the presence of GST-tag both in the construct and in the protease. These characteristics allow us to remove the cleaved tag and the protease at the same time adding GST beads to the sample, minimizing purification steps. In fact, obtaining pure protein using less passages in a reduced time can represent the optimal condition in a purification process. During protein production, a little amount of protein can be lost at each passage and moreover lower time requested for purification can assure protein stability.

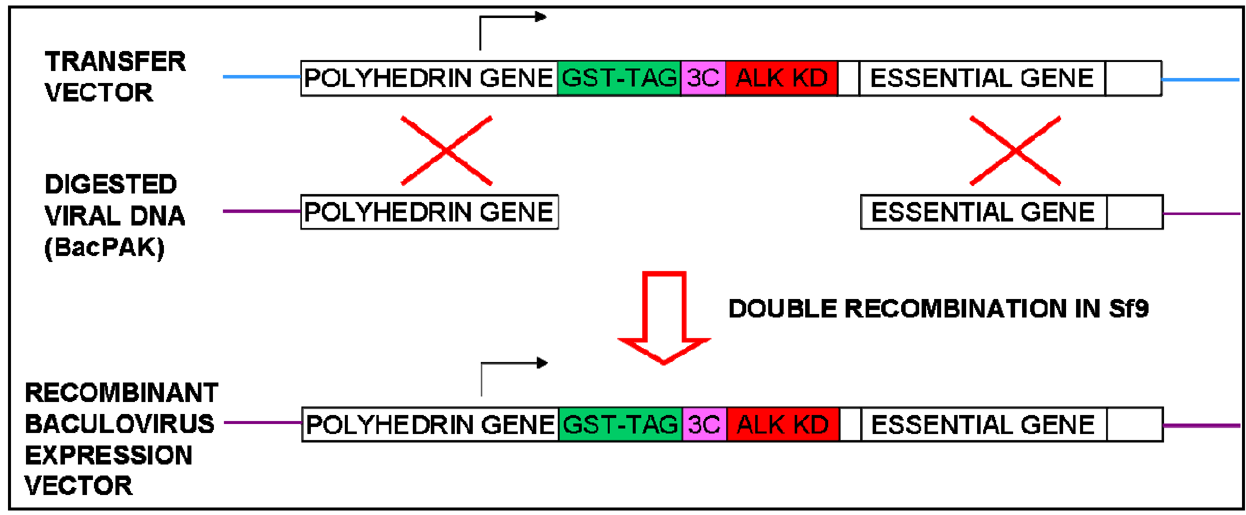


Fig 29_ Production of r-ALK according to the BacPAK Expression System.
r-ALK KD is cloned into the pBacPAK-GST vector producing the transfer vector (pBacPAK-GST-ALK KD). The transfer vector and the viral expression vector (digested BacPAK6) are co-transfected into Sf9 cells for r-Baculovirus production.

Three constructs have been cloned into the pBacPAK vector producing r-ALK 5, r-ALK 6, r-ALK 7 proteins. ALK 5 sequence starts from the residue 1099 of ALK full length including all the C-terminal region (**Fig 30**), as ALK 4. This construct has

been reproduced in a pBacPAK vector after the success of contaminant separation obtained with ALK 4 by SEC; in this case, we were confident to overcome problem of expression using this new vector. ALK 6 and 7 constructs, instead, include a longer amino acid sequence derived from the ALK full length N-terminal region respect to the previous ones. In particular, ALK 6 and ALK 7 sequences start both from the 1064 residue and end at residue 1427 and 1620 respectively (**Fig 31**).

```
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFE
TLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFP
KLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQ/GPLSL
DPFTAGKTSSISDLKEVPRKNITLIRGLGHGAFGEVYEGQVSGMPNDPSPLQVAVKTL
PEVCSEQDELDFLMEALIISKFNHQNIVRCIGVSLQSLPRFILLELMAGGDLKSFLRE
TRPRPSQPSSLAMLDLLHVARDIACGCQYLEENHFIHRDIAARNCLLTCPGPGRVAKI
GDFGMARDIYRASYYRKGGCAMLPVKWMPPEAFMEGIFTSKTDTWSFGVLLWEIFSLG
YMPYPSKSNQEVLEFVTSGGRMDPPKNCPGPVYRIMTQCWQHQPEDRPNFAIILERIE
YCTQDPDVINTALPIEYGPLVEEEEKVPVRPKDPEGVPPLLVSQQAKREEERSPAAPP
PLPTTSSGKAAKKPTAAEVSVRVPRGPAVEGGHVNMAFSQSNPPSELHKVHGSRNKPT
SLWNPTYGSWFTEKPTKKNNPIAKKEPHDRGNLGLEGSCTVPPNVATGRLPGASLLLE
PSSLTANMKEVPLFRLRHFPCGNVNYGYQQQGLPLEAATAPGAGHYEDTILKSKNSMN
QPGP
```

Fig 30_r-ALK 5 amino acid sequence cloned into pBacPAK vector.

In green is the GST-tag sequence, in violet the 3C protease recognition size, in red the ALK kinase domain and in blue is the ALK sequence; the slash indicates the cleavage site.

```
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFE
TLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFP
KLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQ/GPLSL
DPQELQAMQMELQSPEYKLSKLRTSTIMTDYNPNYCFAGKTSSISDLKEVPRKNITLI
RGLGHGAFGEVYEGQVSGMPNDPSPLQVAVKTLPEVCSEQDELDFLMEALIISKFNHQ
NIVRCIGVSLQSLPRFILLELMAGGDLKSFLRETRPRPSQPSSLAMLDLLHVARDIAC
GCQYLEENHFIHRDIAARNCLLTCPGPGRVAKIGDFGMARDIYRASYYRKGGCAMLPV
KWMPPEAFMEGIFTSKTDTWSFGVLLWEIFSLGYMPYPSKSNQEVLEFVTSGGRMDPP
KNCPGPVYRIMTQCWQHQPEDRPNFAIILERIEYCTQDPDVINTALPIEYGPLVEEEE
KVPVRPKDPEGVPPLLVSQQAKREEERSPAAPPPLPTTSSGKAAKKPTAAEVSVRVPR
GPAVEGGHVNMAFSQSNPPSELHKVHGSRNKPTSLWNPTYGSWFTEKPTKKNNPIAKK
EPHDRGNLGLEGSCTVPPNVATGRLPGASLLLEPSSLTANMKEVPLFRLRHFPCGNVN
YGYQQQGLPLEAATAPGAGHYEDTILKSKNSMNQPGP
```

Fig 31_r-ALK 6 and 7 amino acid sequences cloned into pBacPAK vector.
In green is the GST-tag sequence, in violet the 3C protease recognition size, in red the ALK kinase domain and in blue is the ALK sequence; the slash indicates the cleavage site. The ALK 6 and 7 construct ends are shown in light green and black respectively.

ALK 5 PURIFICATION

The ALK 5 protein has been purified according to the on column purification protocol. The increased yield of recovered protein, compared to the old expression system, is evident in the representative experiment shown in **Figure 32**. This protein was unfortunately not stable, as also shown by the degradation bands revealed from the Coomassie analysis. In particular, we observed a partial ALK 5 precipitation during cleavage and a complete protein loss during the following 3 times concentration (**Fig 33**).

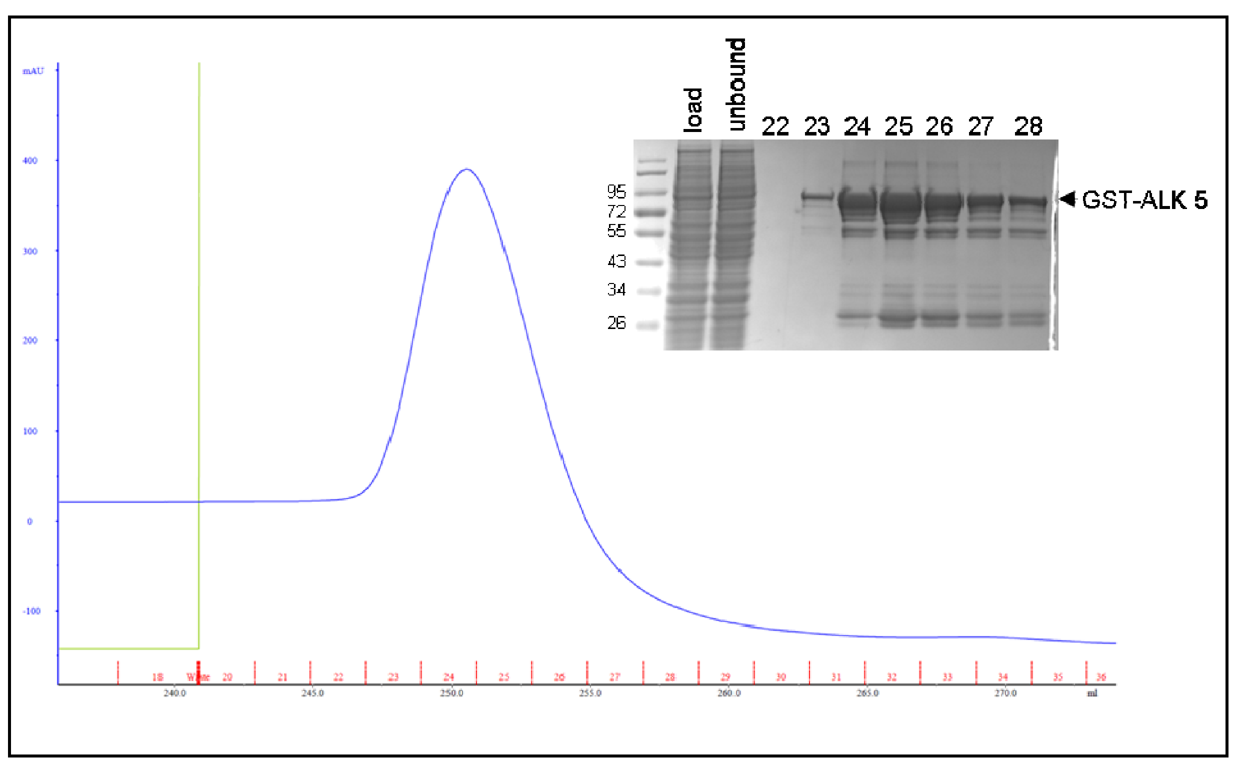


Fig 32_ GST-ALK 5 on column purification.

Zoom of the chromatographic peak and Coomassie Brilliant Blue of ALK 5 purification. Load = sample before purification loaded on FPLC column.

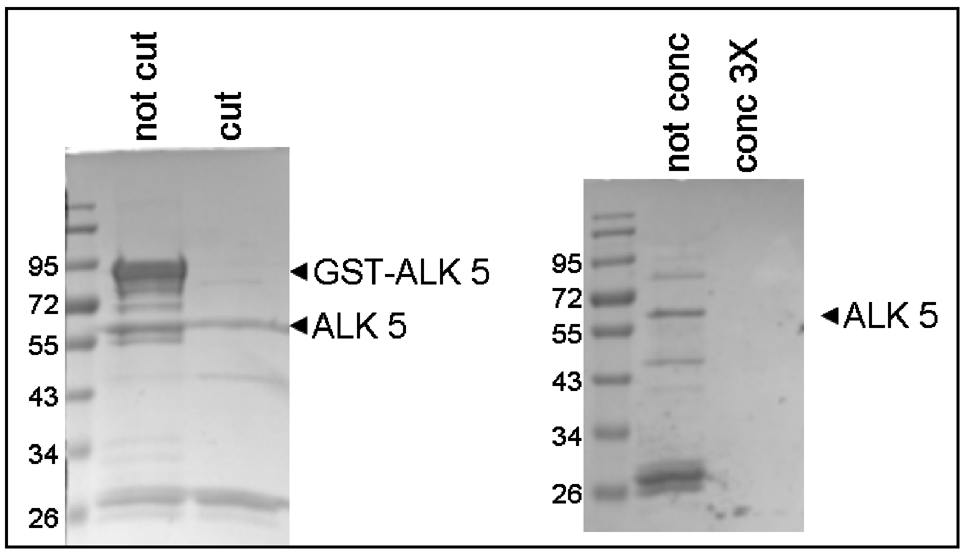


Fig 33_ GST-ALK 5 cleavage and ALK 5 concentration.

Coomassie Brilliant Blue of GST-ALK 5 cleavage (on the left) and ALK 5 concentration (on the right).

Considering these promising results, we started working at 2 new constructs, ALK 6 and ALK 7, supposing a role of the ALK full length N-terminal region in

protein stability. Therefore, we hypothesize that this portion in the new constructs could help to improve protein stability necessary for structural studies.

ALK 6 and ALK 7 PURIFICATION

ALK 6 and 7 studies have been performed in the Structural Biology Laboratory, Cancer Research UK, London. We used the in batch protocol for production of these proteins (**Fig 34**). The on column purification has also been tested without good results. The in batch purification allows the complete separation of r-ALK protein from the tag and the protease thanks to the presence of GST-beads during all the steps. The beads are able to capture all the tagged proteins.

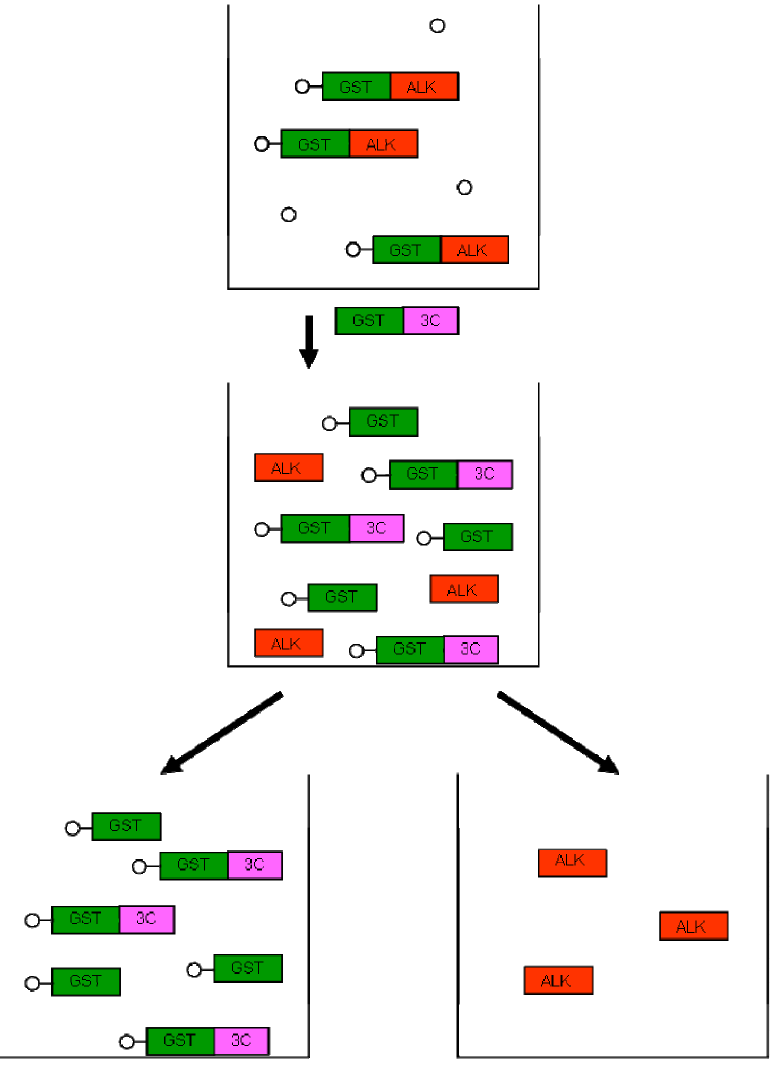


Fig 34_ Scheme of in batch purification for ALK 6 and 7.
GST-ALK is incubated with GST-beads, then GST-3C is added. Finally ALK protein is separated from GST-tag and GST-3C proteins thanks to GST binding to the beads. Balls represent agarose beads.

After purification, r-ALK was concentrated in presence of an ALK known inhibitor (PF-2341066) to increase protein stability and avoid its precipitation. Moreover, the presence of the inhibitor together with the purified protein, during crystallization attempts, permits the crystallization of the compound-protein complex. In addition, the crystal resolution of the complex could give us a lot of data about drug binding and activity.

Protein kinases can have 2 different forms in presence or in absence of ATP corresponding to the activated and inactivated conformation. We do not know which conformation of r-ALK protein is the most stable, so we decided to produce both phosphorylated (P) and non phosphorylated (NON-P) forms and to test them in crystallization trials. The knowledge of both ALK forms (P and NON-P forms) could be very important to understand protein activation.

P-ALK 6 and P-ALK 7 PURIFICATION AND CHARACTERIZATION

We decided to start studying the phosphorylated form of r-ALK (P-ALK), hypothesizing a correlation between protein activity and stability.

ALK 6 and 7 have been purified according to the scheme described in **Figure 35**. GST-beads binding was reduced up to 1 hour avoiding ALK protein degradation mediated by presence of proteases in the cell lysate. The protein was incubated overnight with 5 mM of ATP, after GST-beads binding and before cleavage, in order to obtain the phosphorylated form. In this way, ATP in excess can be easily removed from the final product by more washes before the proteolytic cleavage. The final cleaved sample was incubated with an inhibitor and concentrated for structural tests (**Fig 35**).

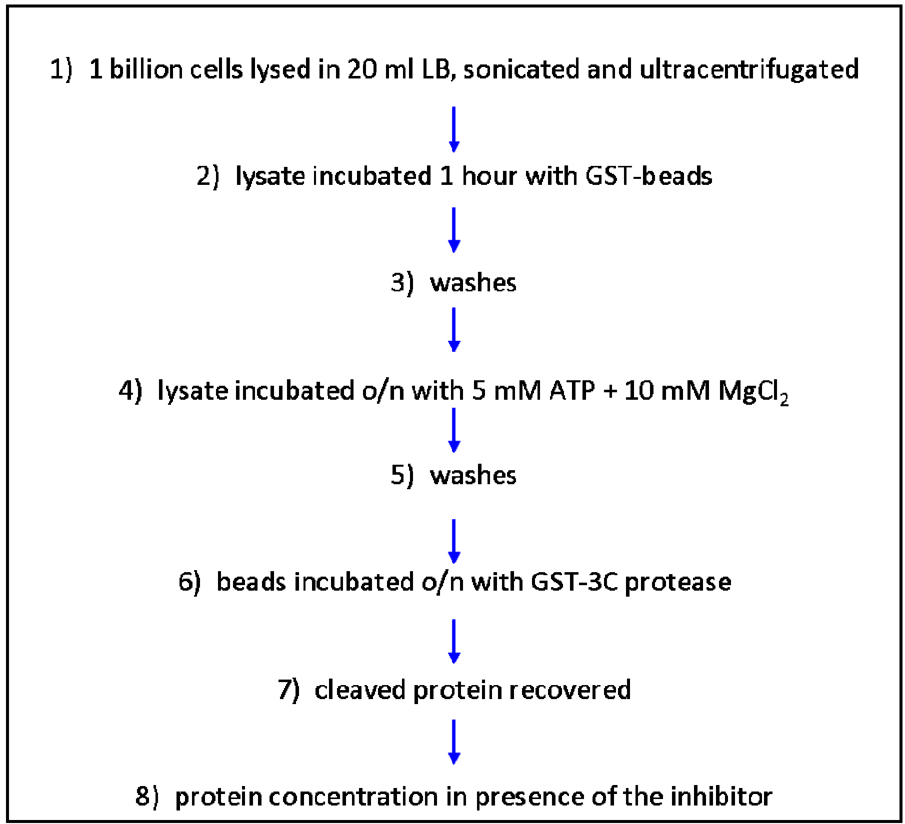


Fig 35_ Scheme of P-ALK purification.

Steps of in batch purification for P- ALK 6 and 7.

The new expression system together with the optimized protein purification procedure allowed us to obtain a greater amount of purified protein compared to that of the old r-ALK constructs. We can also consider level of protein purity: new forms showed an increased purity respect to the previous ones because of low molecular weight contaminants absence (**Fig 36**). Proteins have been concentrated both with and without the PF-2341066 (PF) inhibitor in order to understand if the presence of ALK inhibitor could have an effect on the concentration process. We were able to achieve the same level of protein concentration (5 and 7 mg/mL) either in presence or in absence of PF inhibitor (**Fig 36**).

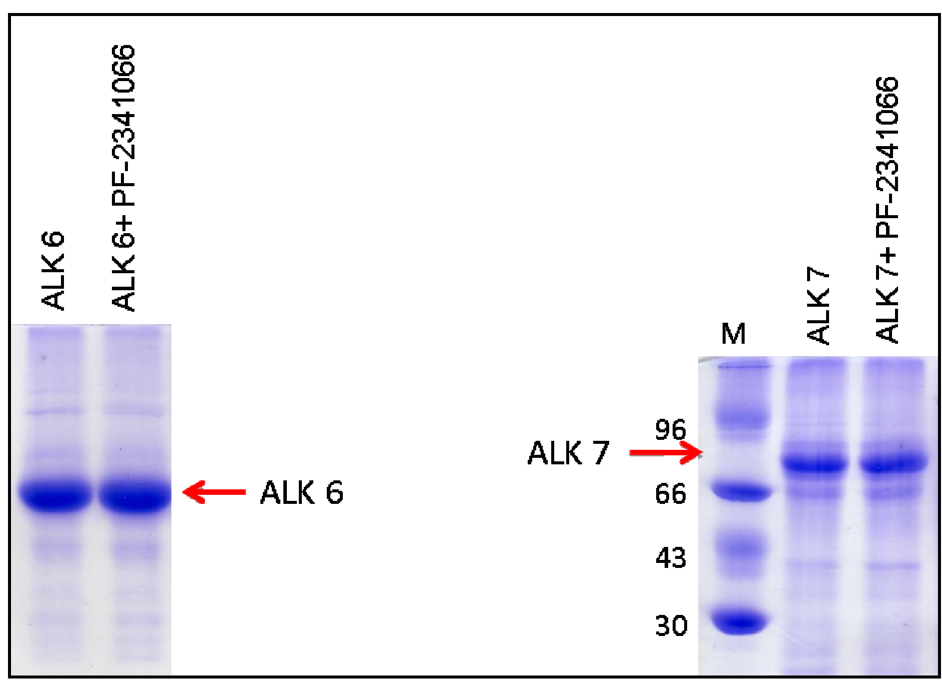


Fig 36_ ALK 6 and 7 purification.

Coomassie Brilliant Blue of purified ALK 6 (on the left) and ALK 7 (on the right), both proteins are concentrated in absence or in presence of the inhibitor PF-2341066. ALK 6 concentration is 7 mg/mL, ALK 7 concentration is 5 mg/mL.

To understand if the proteins were completed phosphorylated during ATP incubation, a cold kinase assay was performed. Phosphorylation of the r-ALK proteins purified in presence of ATP did not significantly increased when the proteins were further incubated with ATP. Therefore, we can assume that proteins were completed phosphorylated after o/n treatment during the purification trial (**Fig 37**).

Fig 37_ ALK 6 and 7 cold kinase assay.

WB anti P-Y of purified P-protein non-treated (-) or treated with ATP. A) ALK 6, B) ALK 7.

Therefore, we were able to obtain higher yield of purified protein eliminating low molecular contaminations. At this point we analyzed the possibility to increase sample quality in order to get proteins suitable for structural studies. First, we

increased number of washes, after GST-ALK binding to the beads before cleavage, to better removed contaminants.

Additionally, we performed two types of chromatography to improve ALK purity:

1. size exclusion chromatography,
2. ion exchange chromatography.

Size exclusion chromatography

The purified protein was loaded on SEC column to analyze the aggregation status and to isolate monomeric non aggregated-protein obtaining a sample composed by an unique species. For the ALK 6 protein, considering the retention volume, we observed that a bigger amount of protein was eluted in the monomeric form (73%), while only 27% of sample resulted as aggregated (**Fig 38**). Differently, considering the ALK 7chromatogram, we observed a very different result: absence of monomeric form of the protein, 70% of aggregated protein and 30% of dimers (**Fig 39**). This could correlate with the presence of the ALK C-terminal region in ALK 7, which probably contribute to protein dimerization.

Therefore, using SEC we isolated the monomeric ALK 6 form, which remained monomeric when loaded again on the same column.

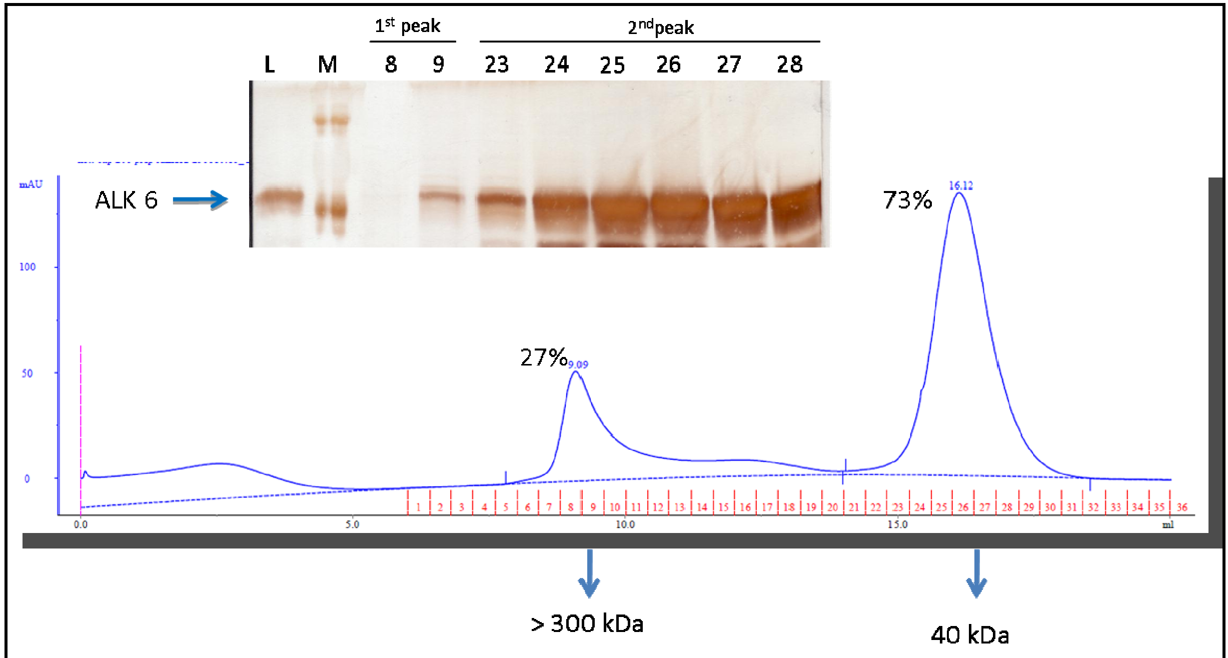


Fig 38_ SEC of ALK 6 purified protein.

Chromatogram of ALK 6 SEC and silver staining, for each peak the percentage and the corresponding molecular weight is shown. L = sample loaded on column; 8,9 = fractions under the first peak; 23-28 = fractions under the second peak.

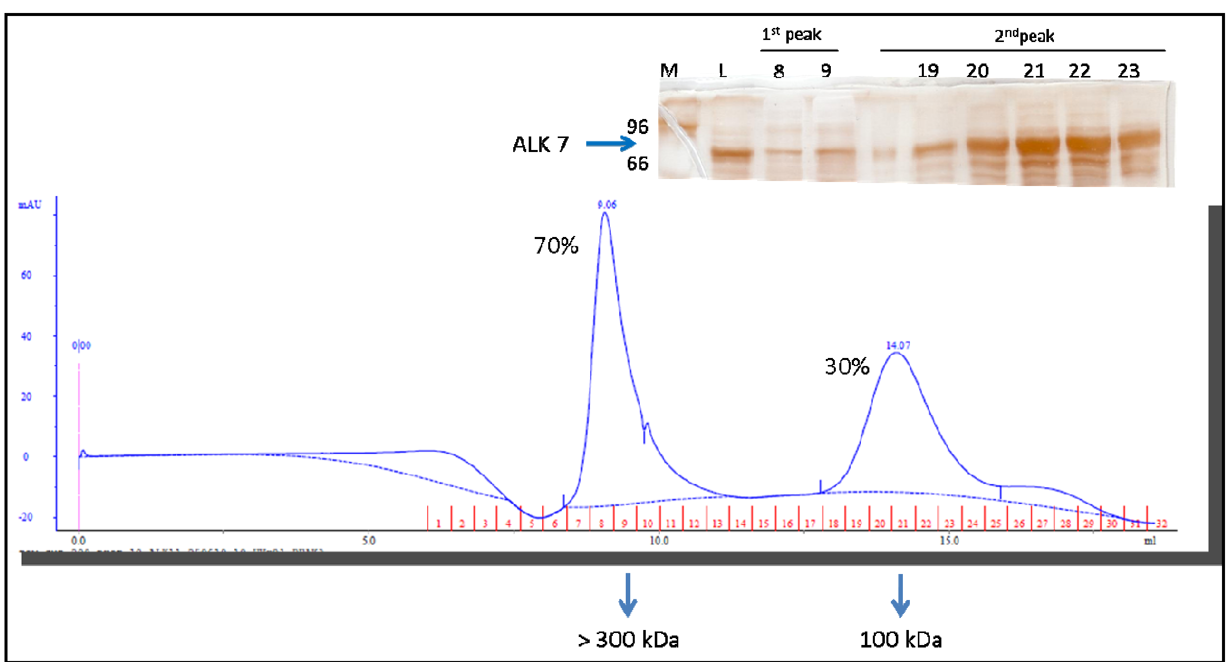


Fig 39_ SEC of ALK 7 purified protein.

Chromatogram of ALK 7 SEC and silver staining, for each peak the percentage and the corresponding molecular weight is shown. L = sample loaded on column; 8,9 = fractions under the first peak; 19-23 = fractions under the second peak.

Ion exchange chromatography

In order to obtain a pure and homogeneous sample we performed IEX chromatography. This procedure permits protein separation according to charge, leading to phosphorylation status analysis of the sample.

Each peak of the IEX chromatogram corresponds to a different phosphorylation status of the protein. Characterization of the ALK activation loop in our laboratory have previously demonstrated that the major autophosphorylation sites are the three tyrosine of the activation loop (Tartari, Gunby, et al. 2008). For this reason, we hypothesized to elute different forms of r-ALK in 4 different peaks by IEX chromatography, respectively corresponding to: 1) all non-P form, 2) one phosphorylated tyrosine form, 3) two phosphorylated tyrosines form and 4) all 3 phosphorylated tyrosines.

For both P-ALK 6 and 7, the IEX chromatography revealed a single peak of eluted protein as shown in a representative experiment (**Fig 40-41**). In particular, considering the ALK 7 characterization, analysis of eluted fractions performed by a Coomassie gel demonstrated the absence of ALK 7 protein in correspondence to the first peak. ALK 7 was instead present in fractions eluted under the second higher peak (**Fig 41**). This result suggests that purified sample is composed by protein molecules belonging to the same phosphorylated species.

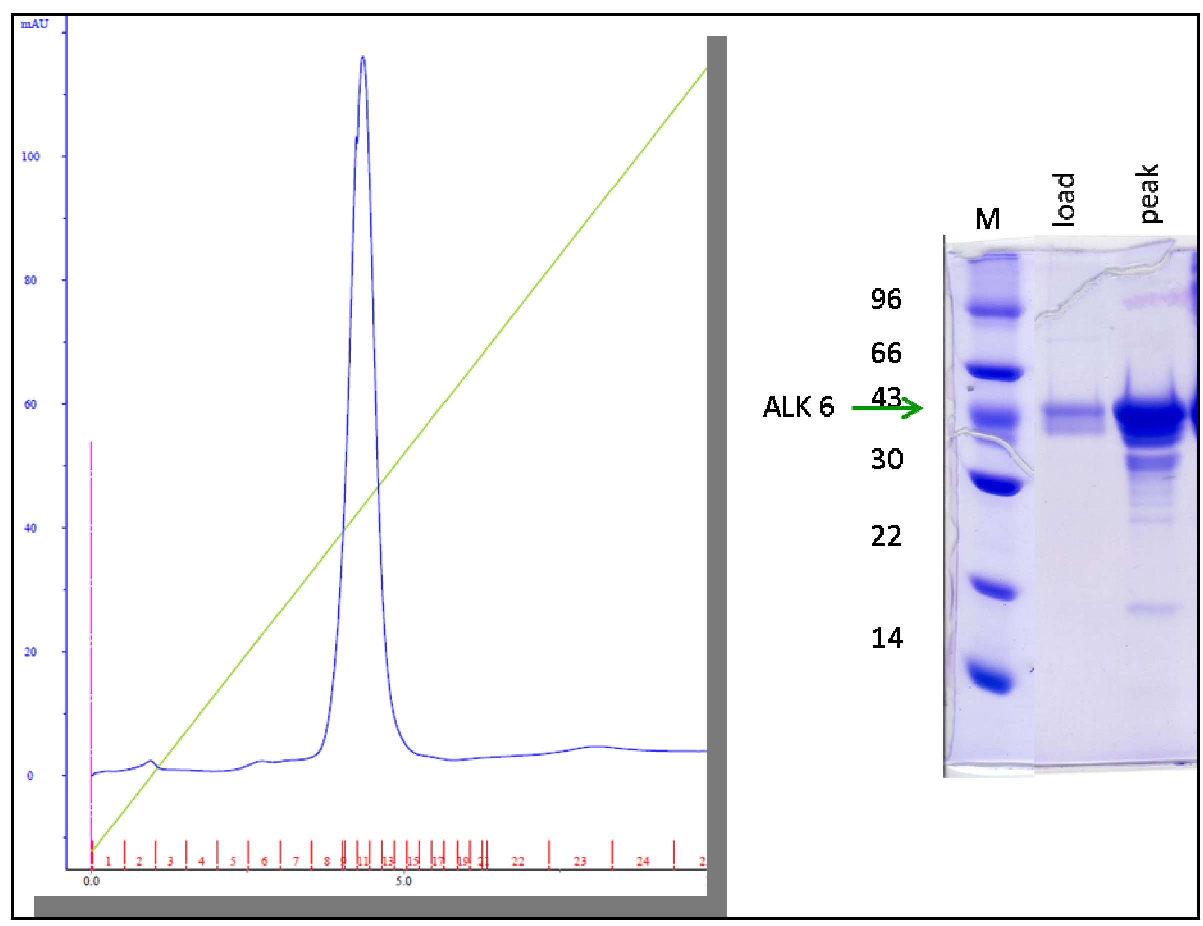


Fig 40_ IEX chromatography of P-ALK 6 protein.

Chromatogram of P-ALK 6 protein IEX and Coomassie Brilliant Blue. Leaded = sample loaded on the column, peak = pooled and concentrated isolated protein.

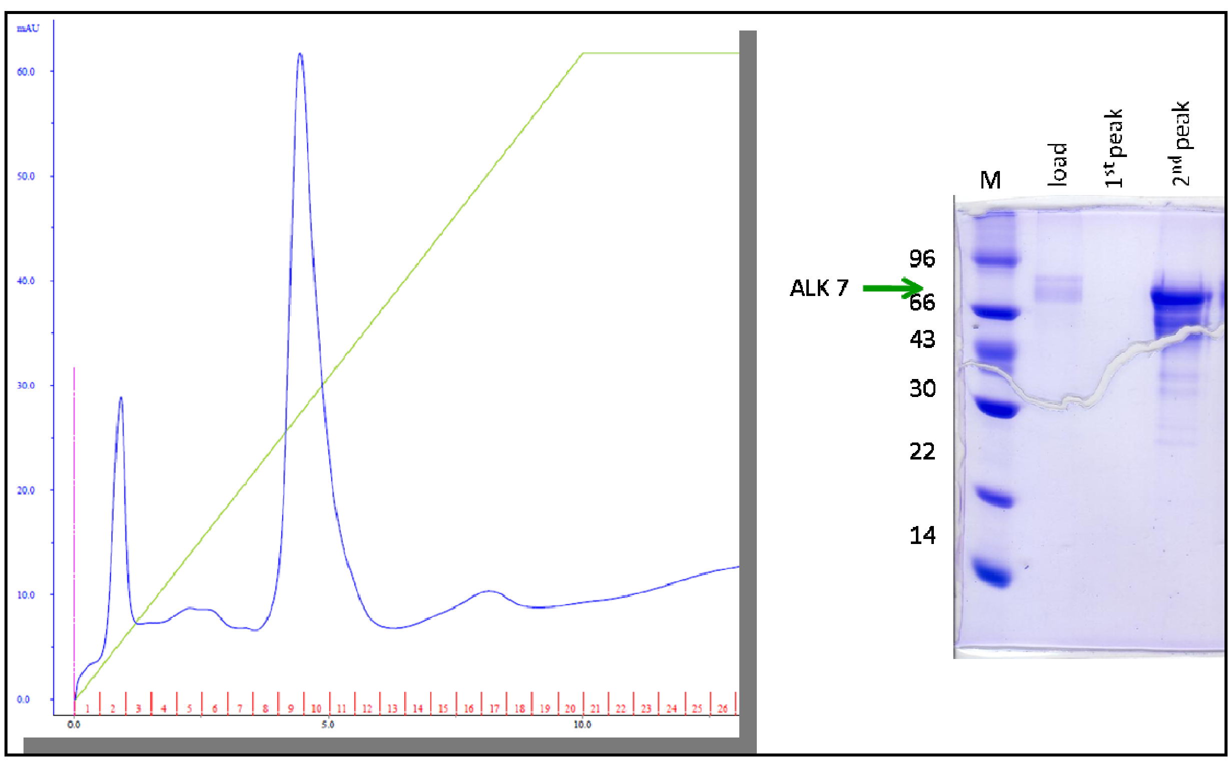


Fig 41_ IEX chromatography of P-ALK 7 protein.

Chromatogram of P-ALK 7 protein IEX and Coomassie Brilliant Blue. Leaded = sample loaded on the column, 1st peak = pooled and concentrated protein eluted under the first peak, 2nd peak = pooled and concentrated protein eluted under the second peak.

Result of the kinase assay demonstrated that loaded protein was completely phosphorylated, so, we can assume that the single peak corresponds to the 3 tyrosine phosphorylated form (**Fig 37**).

NON-P ALK6 and NON-P ALK 7 PURIFICATION AND CHARACTERIZATION

In order to characterize the inactive form of ALK kinase domain we also produced NON-P forms of ALK 6 and 7 recombinant proteins. The protocol used in this case was very similar to the one described for P-ALK purification. No ATP was added and the final cleaved sample was incubated with an inhibitor and concentrated for structural tests (**Fig 42**).

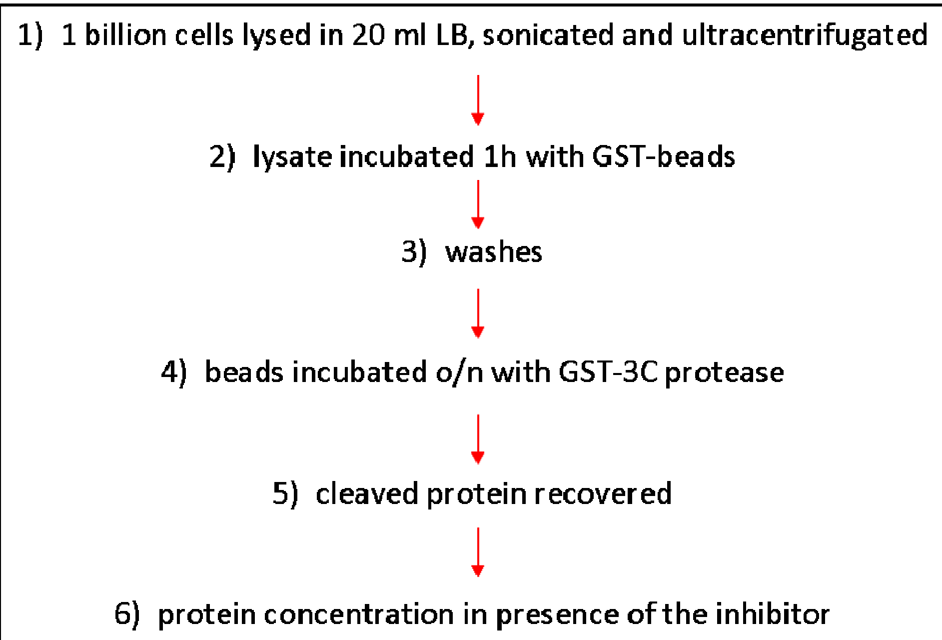


Fig 42_ Scheme of NON-P ALK purification.

Steps of the in batch purification for NON-P ALK 6 and 7.

Before protein concentration, the cleaved sample was loaded on IEX column to analyze the protein phosphorylation in absence of ATP incubation. IEX chromatography was chosen as protein purification procedure considering its success in pure protein isolation in P-ALK studies. In fact, IEX chromatography results demonstrated elution of an homogeneous sample suggesting high probability of protein crystallization.

In particular, IEX chromatography of ALK 6 protein revealed two peaks: the first corresponding to non phosphorylated protein; and the second smaller peak corresponding to the phosphorylated form of ALK 6. Coomassie gel shows the protein recovered under the first peak and concentrated up to 7 mg/mL in presence of PF, while protein eluted under the second peak was too small for Coomassie sensitivity (**Fig 43**).

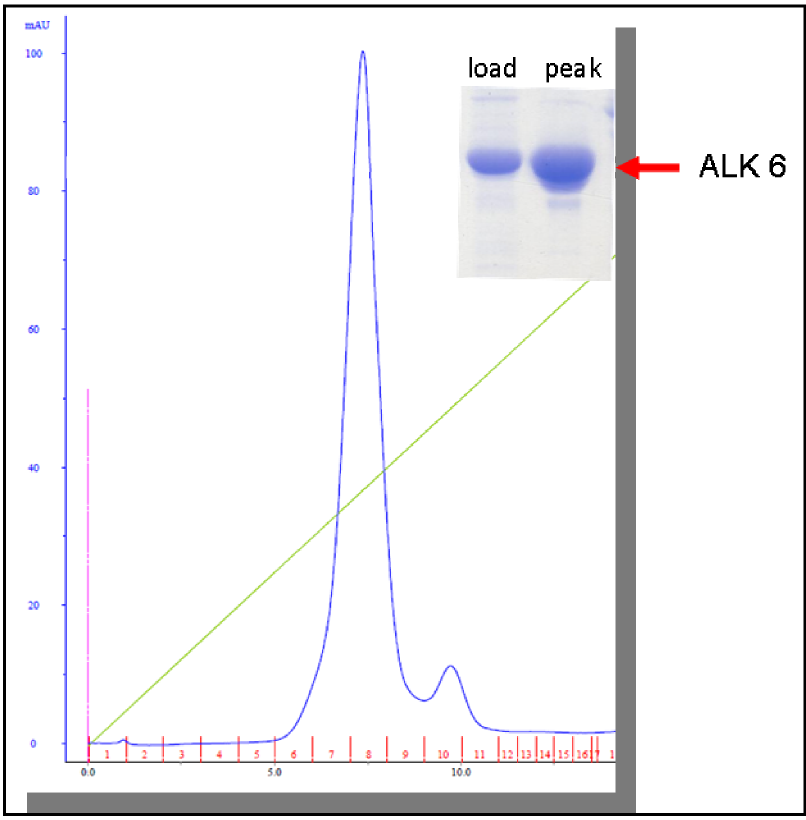


Fig 43_ IEX of ALK 6 protein.

Chromatogram of ALK 6 protein IEX and Coomassie Brilliant Blue of leaded sample on the column and of the pooled and concentrated protein under the first peak.

Similarly, IEX chromatography of ALK 7 revealed elution of the target protein under two peaks: a first higher peak and a second smaller one. We concentrated the first peak in presence of PF up to 6 mg/mL (**Fig 44**).

In conclusion, performing IEX chromatography, we obtained enough NON-P r-ALK forms amount suitable for structural studies.

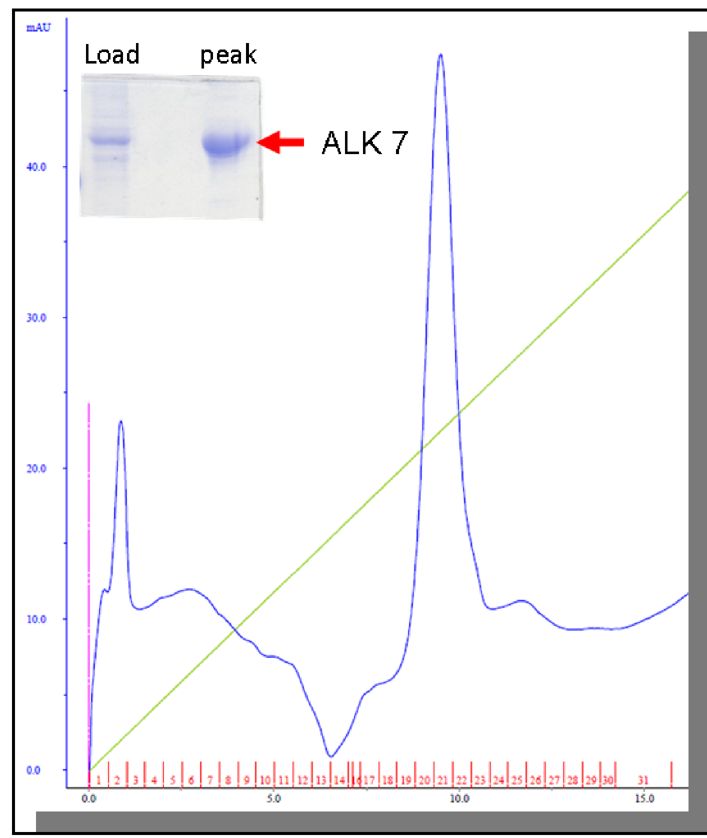


Fig 44_ IEX of ALK 7 protein.

Chromatogram of ALK 6 protein IEX and Coomassie Brilliant Blue of leaded sample on the column and of the pooled and concentrated protein under the first peak.

Study of phosphorylation status of r-ALK forms

To best characterize r-protein phosphorylation, the two peaks resulted from each NON-P ALK IEX (**Fig 43, 44**), were separately analyzed by WB.

The first result was the absence of phosphorylation both in first and second ALK 6 and ALK 7 peaks, as shown by WB (**Fig 45, 46**). This is a really unexpected result considering their separation by IEX (**Fig 43, 44**). Not phosphorylated protein, coming from the first peak of each IEX chromatogram, can be phosphorylated adding ATP, as shown by the time course phosphorylation. The complete phosphorylation was obtained after o/n treatment both for ALK 6 and 7 because incubation with ATP for longer time did not confer a signal increase in WB. Moreover, phospho-protein can be de-phosphorylated adding phosphatase. The phosphatase was added after dialysis in order to remove the ATP still present in the sample (**Fig 45, 46**). So, looking at these results, we can conclude that purified cleaved protein was capable of autophosphorylation and de-phosphorylation; this demonstrated protein activity.

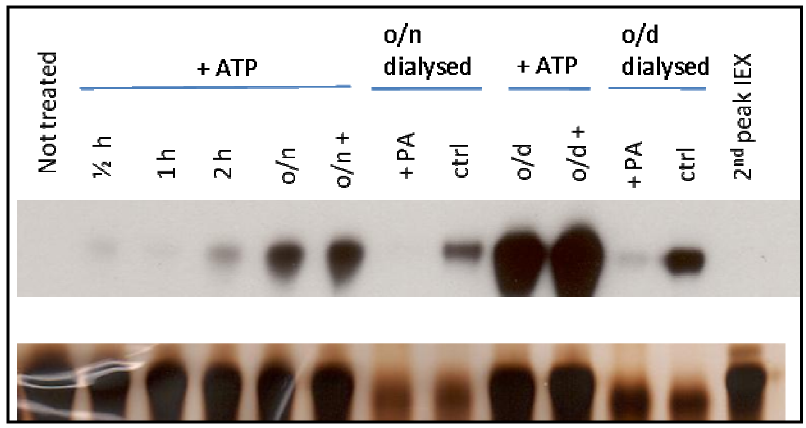


Fig 45_ Phosphorylation analysis of ALK 6 protein.

WB anti P-Y (upper panel) and Silver Staining (lower panel) of ALK 6. 10 μg of protein are treated with 5 mM ATP and 10 mM $MgCl_2$ for 1/2, 1, 2, 13 (o/n), 18 (o/n+), 26 (o/d) and 32 hours. After phosphorylation, both o/n and o/d treated proteins are dialyzed o/d (called o/n dialysed and o/d dialysed respectively) and incubated o/n with phosphatase (PA) or buffer (ctrl). Not treated = protein from IEX first peak , 2nd peak IEX = protein from IEX second peak.

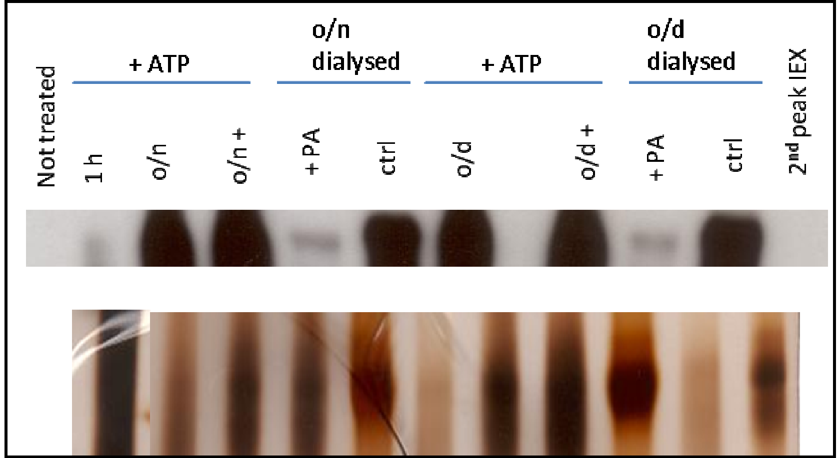


Fig 46_ Phosphorylation analysis of ALK 7 protein.

WB anti P-Y (upper panel) and Silver Staining (lower panel) of ALK 7. 10 μg of protein are treated with 5 mM ATP and 10 mM $MgCl_2$ for 1, 13 (o/n), 18 (o/n+), 26 (o/d) and 32 hours. After phosphorylation, both o/n and o/d treated proteins are dialyzed o/d (called o/n dialysed and o/d dialysed respectively) and incubated o/n with phosphatase (PA) or buffer (ctrl). Not treated = protein from IEX first peak , 2nd peak IEX = protein from IEX second peak.

Mass spectrometry analysis of r-ALK forms

After the lack of differences in WB analysis, the separated peaks by IEX have been analyzed by mass spectrometry to understand the reason for the separation. The analysis has been performed by Cancer Research UK Mass spectrometry Facility.

ALK 6 analysis displayed lack of any type of phosphorylation in both first and second peak. The mass spectrometry analysis suggested that protein separation could be caused by modifications normally present in proteins, such as oxidations and carbamidamethylation, instead of phosphorylation.

ALK 7 second peaks analysis instead revealed the presence of S-1437 phosphorylation, which could be responsible for separation. This serine is not present in ALK 6 sequence.

ALK 6 L1196M PURIFICATION AND CHARACTERIZATION

The L1196M mutation is an important ALK mutation because it has been found in NSLC patients resistant to the PF-2341066 inhibitor. The mutation is localized at the gatekeeper, near the ATP binding pocket. In particular, the amino acid substitution from leucine to methionine, which has a greater bulk, does not allow the inhibitors to bind inside the ATP pocket any more. So, the study of binding between this mutated form and different ALK inhibitors could help to find more active inhibitors and to understand their activity. Therefore, the ALK 6 construct have been modified by the insertion of the L1196M mutation (called ALK 6 L1196M) and again, for this form production, it has been cloned into the pBacPAK vector for expression in Sf9 cells (**Fig 47**).

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFE
TLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFP
KLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQ/GPLSL
DPQELQAMQMELQSPEYKLSKLRTSTIMTDYNPNYCFAGKTSSISDLKEVPRKNITLI
RGLGHGAFGEVYEGQVSGMPNDPSPLQVAVKTLPEVCSEQDELDFLMEALIISKFNHQ
NIVRCIGVSLQSLPRFILMELMAGGDLKSFLRETRPRPSQPSSLAMLDLLHVARDIAC
GCQYLEENHFIHRDIAARNCLLTCPGPGRVAKIGDFGMARDIYRASYYRKGGCAMLPV
KWMPPEAFMEGIFTSKTDTWSFGVLLWEIFSLGYMPYPSKSNQEVLEFVTSGGRMDPP
KNCPGPVYRIMTQCWQHQPEDRPNFAIILERIEYCTQDPDVINTALPIEYGPLVEEEE
KVPVRPKDPEGVPPLLVS

Fig 47_ r-ALK 6 MUT (L1196M) amino acid sequence cloned into pBacPAK vector.

In green is the GST-tag sequence, in violet the 3C protease recognition size, in red the ALK kinase domain and in blue is the ALK sequence; the slash indicates the cleavage site. The L1196M mutation is shown in black.

The protein has been purified according to the previous protocol (**Fig 42**) without ATP addition to study the NON-P ALK 6 L1196M form. Surprisingly, the resulted purified protein has shown tyrosine phosphorylation also in absence of ATP incubation. In particular, the phosphorylation signal from the non-treated protein was stronger than the signal from the same purified protein treated with ATP. Moreover, the phosphorylation was the same both in presence and in absence of ATP, also when a kinase assay was performed as positive control: protein treated with ATP at 30℃ to obtain complete phosphorylation. The Coomas sie of the purified protein is shown in the first lane (**Fig 48**). In this case we checked gel loading by Ponsueau staining.

A possible explanation for the ALK 6 L1196M phosphorylation could be linked to the mutation itself: the amino acid substitution could make the protein more active, so more easily phosphorylable by the ATP present in the Sf9 cells.

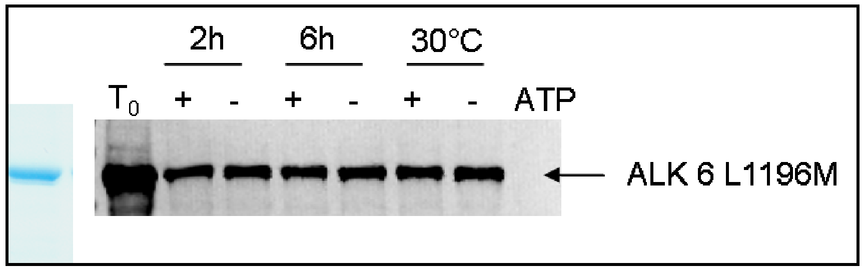


Fig 48_ Analysis of purified ALK 6 L1196M phosphorylation.

Coomassie of purified ALK 6 L1196M, on the left, and WB anti P-Y of purified ALK 6 L1196M phosphorylation test on the right. Protein has been treated with or without ATP for 2 or 6 hours at 4℃ and for 10 minutes at 30℃. T_0 = non treated protein.

The P-ALK 6 L1196M protein has been easily concentrated in presence of NVP-TAE684, a known ALK mutant inhibitor.

To obtain the NON-P ALK 6 L1196M form, 1 μM of NVP-TAE684 was added to Sf9 cells during infection. NVP-TAE684 should be able to enter into insect cells and bind the r-ALK preventing protein phosphorylation. In fact, the inhibitor was able to completely de-phosphorylate the protein (**Fig 49**). A different new ALK inhibitor, R500B developed in our laboratory, did not give the same result in terms of de-phosphorylation: ALK is still phosphorylated also at higher inhibitor concentrations (**Fig 50**). Therefore, NVP-TAE684 was used to obtain NON-P ALK 6 L1196M.

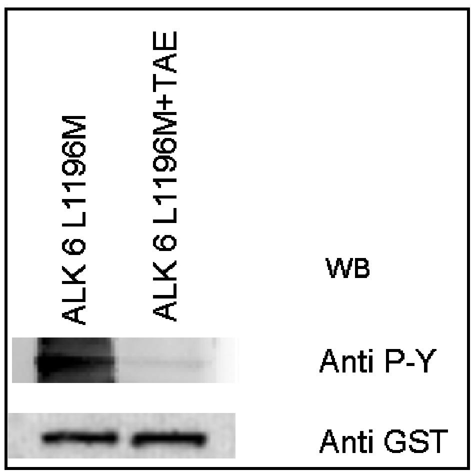


Fig 49_ ALK 6 L1196M phosphorylation in presence of NVP-TAE684.

WB anti P-tyrosine and anti GST as loading control of Sf9 cells infected with Baculovirus expressing the ALK 6 L1196M protein. ALK 6 L1196M = lysate of infected cells, ALK 6 L1196M+TAE = lysate of infected cells in presence of NVP-TAE684.

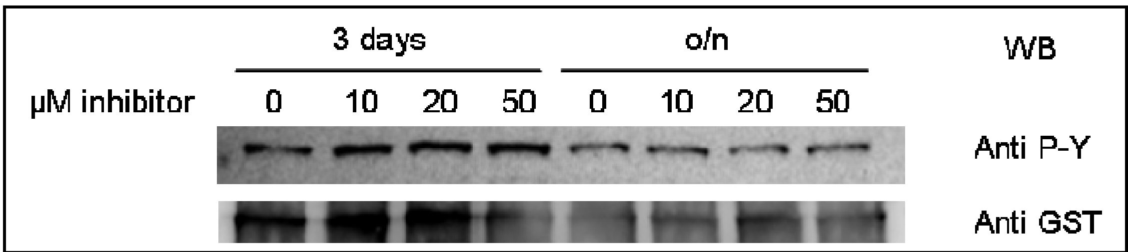


Fig 50_ ALK 6 L1196M de-phosphorylation test in presence of R500B.
WB anti P-tyrosine and anti GST as loading control of Sf9 cells infected with Baculovirus expressing the ALK 6 L1196M protein. Cells have been treated with 0, 10, 20 and 50 μM of inhibitor for 3 days during infection (3 days) or added just the night before the harvesting (o/n).

The NON–P ALK 6 L1196M was purified according to the optimized procedure (**Fig 42**) and concentrated in presence of NVP-TAE684. The obtained protein was finally stable, as the concentration up to mg/mL range was possible (**Fig 51**).

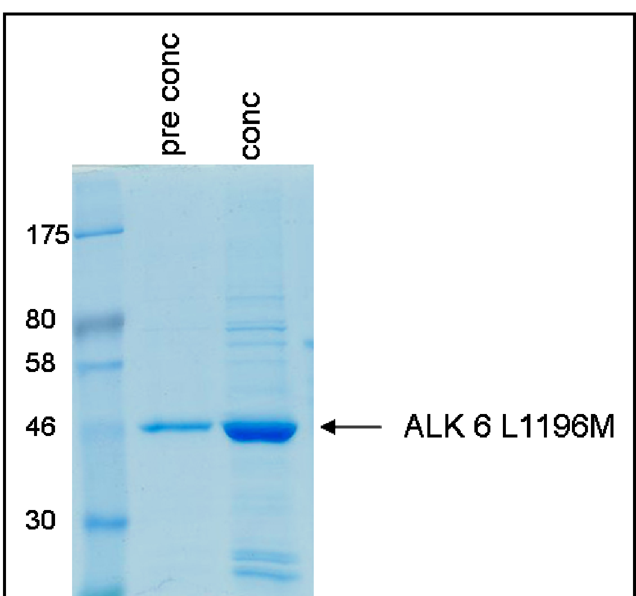


Fig 51_ NON–P ALK 6 L1196M purification.
Coomassie of NON–P ALK 6 L1196M purification; pre conc = purified protein before concentration, conc = protein concentrated 20 times.

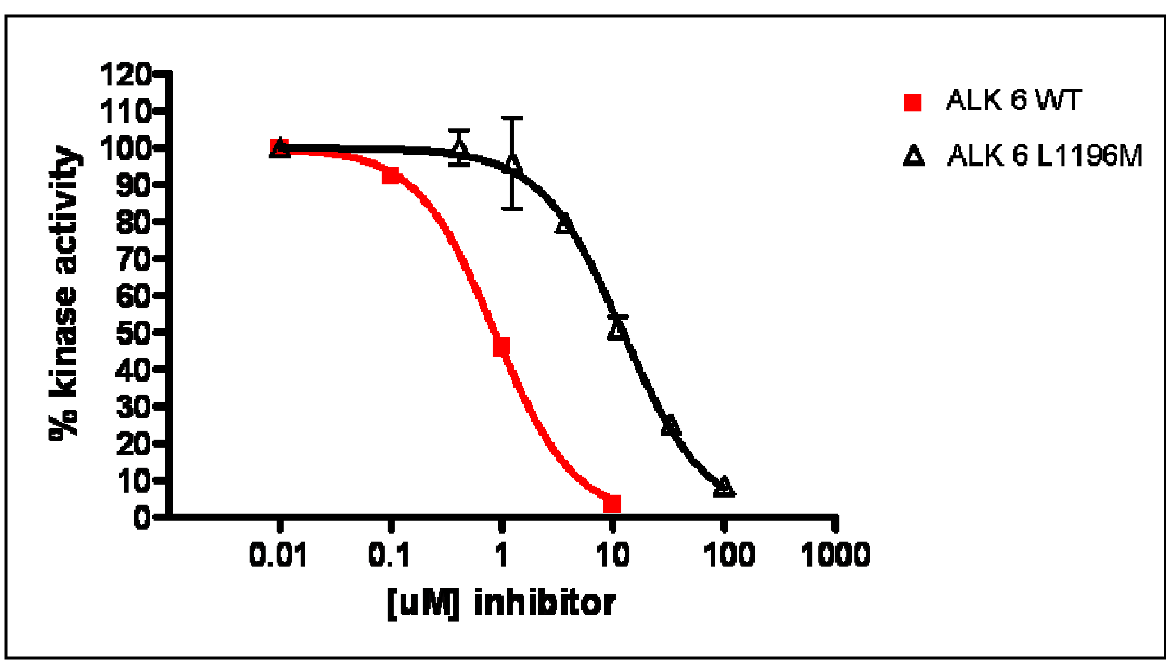
Preliminary crystallization trials for the P and NON-P ALK 6 L1196M proteins structural determination are ongoing.

r-ALK ACTIVITY

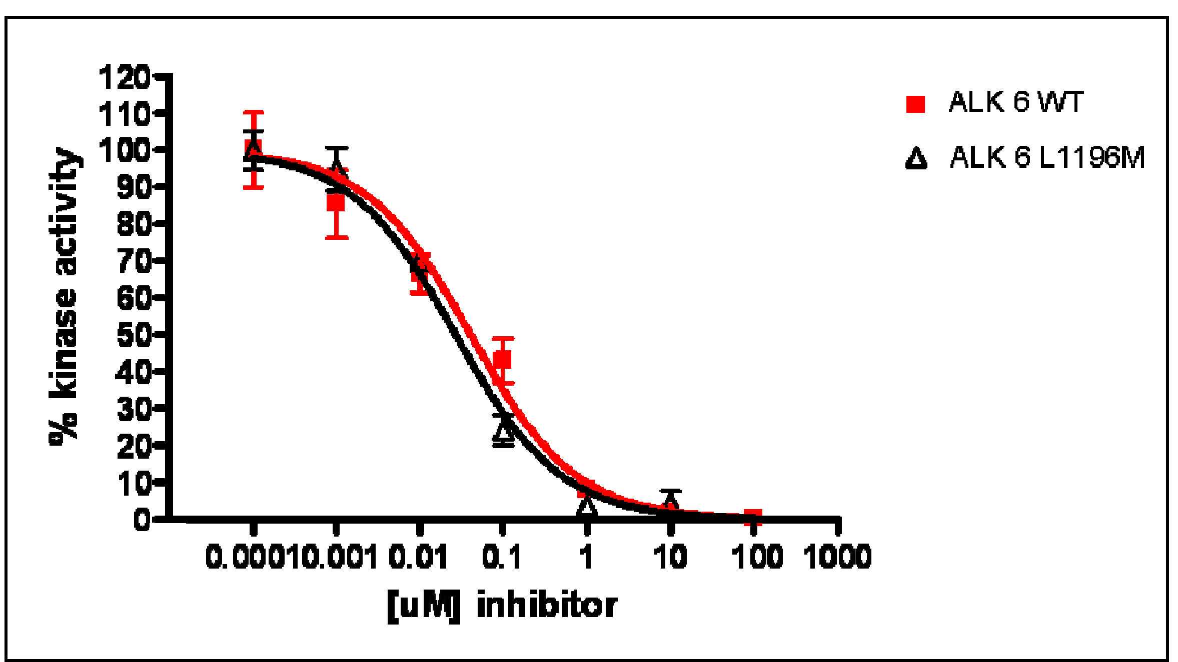
The purified r-ALK WT (ALK 6) and mutant form (ALK 6 L1196M) have been used to perform ELISA kinase assay to test their activity in presence of different ALK inhibitors. In this way we can also test the capacity of a specific compound to block the protein oncogenic activity.

Proteins activity in presence of two known ALK inhibitors, PF-2341066 (**Fig 52A**) and NVP-TAE684 (**Fig 52B**), and two new compounds, R500B (**Fig 52C**) and R458 (**Fig 52D**), have been analyzed. In the first experiment, the EC_{50} (the concentration of a drug that gives half-maximal response) in presence of PF-2341066 is 0.9 μM for the WT and 12 μM for the mutant underlining the inhibitor efficiency against the WT (**Fig 52A**). NVP-TAE684 is instead active both against the WT, EC_{50} 0.04 μM, and against the mutant, EC_{50} 0.03 μM, in a similar way (**Fig 52B**). Regarding the two new compounds, R500B has shown activity both against the WT, with an EC_{50} 0.1 μM, and against the mutant, EC_{50} 0.08 μM (**Fig 52C**); while R458 is only active against the WT, EC_{50} 20 μM, but it has no effect against the mutant form where the EC_{50} is more than 100 μM (**Fig 52D**).

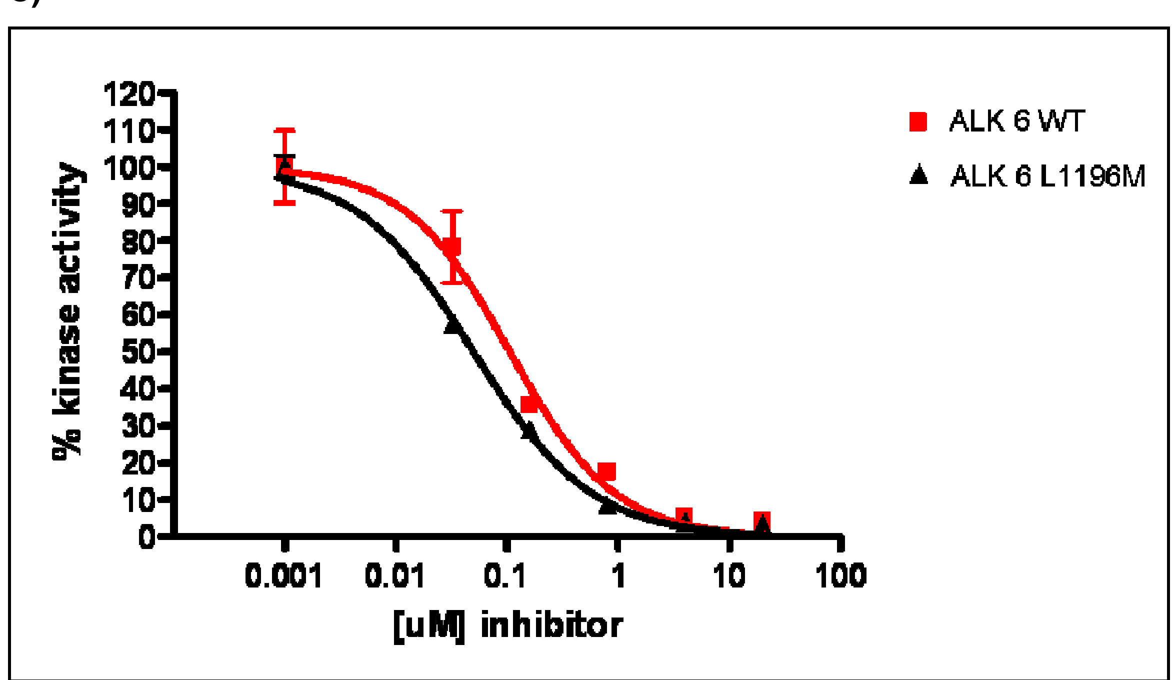
A)



B)



C)



D)

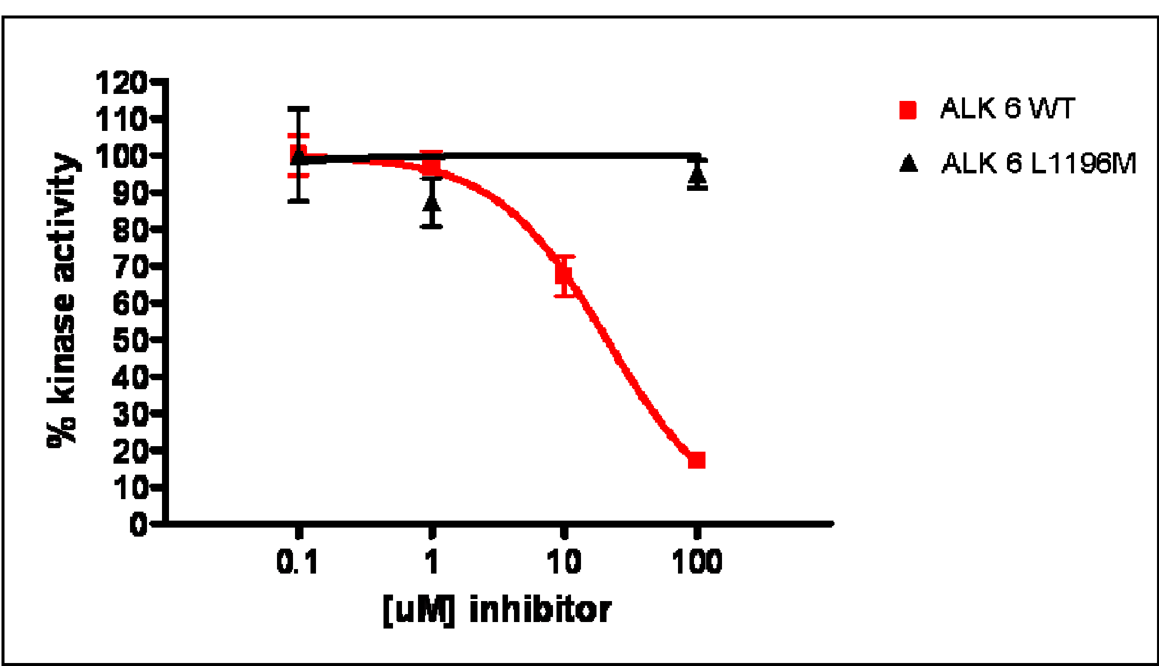


Fig 52_ ELISA kinase assay for r-ALK activity.

Logarithmic curves of ELISA kinase assay of ALK 6 WT (in red) and ALK 6 L1196M (in black) proteins in presence of PF-2341066 (A), NVP-TAE684 (B), R500B (C) and R458 (D). In x-axis is the inhibitor μM concentration, in y-axis is the percentage of the kinase activity.

In conclusion, the purified proteins can be inhibited by specific ALK inhibitors and we were able to find new drugs active against the recombinant proteins: the R500B inhibits both the WT and the mutant form while R458 inhibits only the WT one.

r-ALK CRYSTALLIZATION

Protein crystallization is a very delicate process. There is a slight equilibrium between crystal formation and protein precipitation. Purified protein is dissolved in an aqueous buffer containing a precipitant at a concentration just below that necessary to protein precipitation. Protein seeded on plates need purity >95%, with a concentration in terms of mg/mL range (~10 mg/mL) to have chance to crystallize. Moreover, finding optimal condition for crystal growth can be very hard: the effect of pH on precipitation in presence of a specific precipitant must be determined, different temperatures (at 4℃ or at 20℃) and prot ein concentrations (5-10 mg/mL) must be considered and the experiments must be repeated with different precipitating agents (for example Ammonium sulfate, glycerol, MPD, PEG, Isopropanol, Tris, Hepes, NaCl).

ALK 6 and 7 were characterized by enough level of purity, solubility, concentration, stability, biological activity and homogeneity for crystallization. So, both proteins in their P and NON-P forms have been used for crystallization trials performed in the Structural Biology Laboratory, Cancer Research UK, London.

Crystallization attempts have been performed using the vapour diffusion method in which nanoliters of purified protein are mixed together with a precipitating solution, usually composed by salt, buffer and precipitant agents; the “drop” is located in a well near a reservoir containing the precipitating solution alone (**Fig 53**). The crystal should appear from the well after the precipitant concentration increases to a level optimal for crystallization thanks to water vaporization from the drop and transfer to the reservoir.

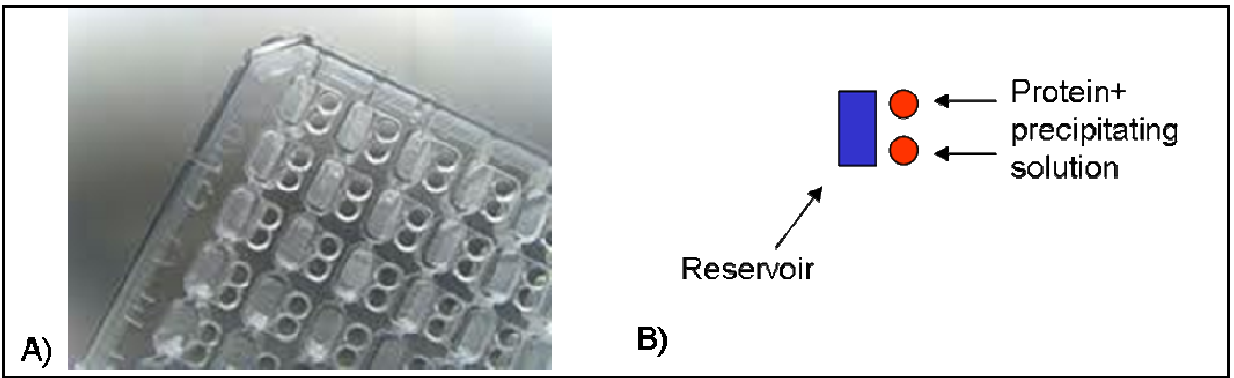


Fig 53_ 96-well sitting drop plate.

A) Imagine of a 96-well sitting drop plate. B) Scheme of the crystallization plate; the reservoir containing the precipitating solution is shown in blue, while the wells containing the drops are shown in red.

r-ALK STABILITY STUDY

Protein stability is very important to avoid protein aggregation and precipitation during concentration. Therefore, finding the optimal condition for protein stability can help to increase protein concentration, a key point in X-ray crystallography.

The r-ALK proteins have been concentrated up to 1mg/mL in complex with PF-2341066 and tested for stability using a thermal denaturing assay. This assay is based on a fluorescent dye, Sypro Orange, which is able to bind hydrophobic regions of the protein becoming fluorescent. When the temperature rises and the protein undergoes thermal unfolding, Sypro Orange fluorescence signal increases. In this way the melting temperature (Tm) of protein in presence of different buffers can be analyzed

We tested 16 buffers to find out the condition in which r-ALK shows higher stability, that is higher Tm. We tested parameters involved in protein stability : DTT concentration, pH value and NaCl concentration. For P-ALK 6 the Tm are shown in the following histogram (**Fig 54**). Surprisingly, the higher Tm corresponds to condition 9, the buffer without NaCl. So, P-ALK 6 in presence of PF-2341066 has a better stability in presence of 50 mM Tris pH 8+ 1 mM DTT+ 0.5 mM EDTA+ 0 mM NaCl. For this reason, this buffer was the final buffer used in P-ALK 6 purification.

The thermal denaturing assay results for P-ALK 7, NON-P ALK 6 and 7 are not reported because no significant differences have been shown.

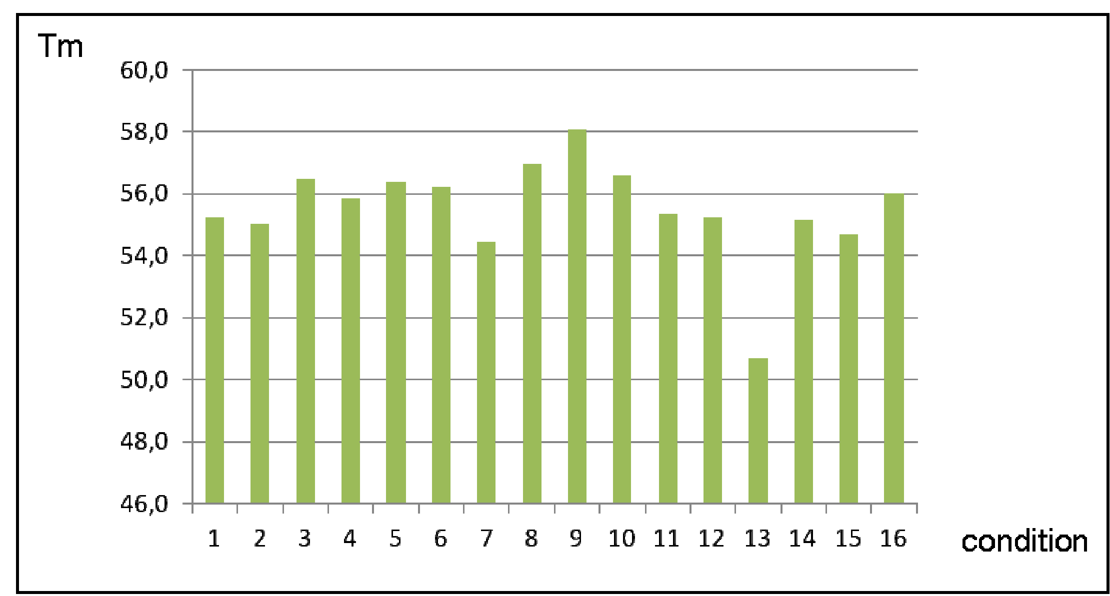


Fig 54_ P-ALK 6 stability test.

*Histogram of P-ALK 6 Tm in different conditions, Tm have been analyzed by thermal denaturing assay. Cond **1** = 50mM Tris pH 8+100mM NaCl+0,5mM EDTA+**2mM DTT**; cond **2** = 50mM Tris pH 8+100mM NaCl+0,5mM EDTA+**3mM DTT**; cond **3** = 50mM Tris pH 8+100mM NaCl+0,5mM EDTA+**0mM DTT**; cond **4** = 50mM **Tris pH 7**+100mM NaCl+0,5mM EDTA+1mM DTT; cond **5** = 50mM **Tris pH 7,5**+100mM NaCl+0,5mM EDTA+1mM DTT; cond **6** = 50mM **Tris pH 8,5**+100mM NaCl+0,5mM EDTA+1mM DTT; cond **7** = 50mM **Tris pH 9**+100mM NaCl+0,5mM EDTA+1mM DTT; cond **8** = 50mM Tris pH 8+**50mM NaCl**+0,5mM EDTA+1mM DTT; cond **9** = 50mM Tris pH 8+**0mM NaCl**+0,5mM EDTA+1mM DTT; cond **10** = 50mM Tris pH 8+**150mM NaCl**+0,5mM EDTA+1mM DTT; cond **11** = 50mM Tris pH 8+**200mM** NaCl+0,5mM EDTA+1mM DTT; cond **12** = 50mM Tris pH 8+**250mM NaCl**+0,5mM EDTA+1mM DTT; cond **13** = 50mM Tris pH 8+**300mM NaCl**+0,5mM EDTA+1mM DTT; cond **14** = 50mM Tris pH 8+**400mM NaCl**+0,5mM EDTA+1mM DTT; cond **15** = 50mM Tris pH 8+**500mM NaCl**+0,5mM EDTA+1mM DTT; cond **16** = 50mM Tris pH 8+100mM NaCl+0,5mM EDTA+1mM DTT.*

CRYSTALLIZATION TRIALS

We used different crystallization kits ($AmSO_4$ Suite, Classic Lite Suite, PEGs Suite, MPD Suite, Ph Clear Suite, Ph Clear II Suite, PACT Suite, JCSG Core Suite I, JCSG Core Suite II, JCSG Core Suite III, JCSG Core Suite IV, HR2-086, HR2-098, HR2-130, HR2-133, HR2-134, HR2-136, HR2-137, HR2-139, Structure Screen 1&2, Clear Strategy Screen I, Clear Strategy Screen II) (**Appendix 1**) to test about 10000 conditions for each protein. Spheruloids, typical early phase crystal growth formations, appeared repeatedly in presence of PEG precipitant. This suggested the need of condition optimization in presence of PEG to obtain crystal. So, we also tested the effect of PEG in combination with different pH levels (PEG and pH screening) and the effect of PEG in combination with different NaCl concentrations (PEG and NaCl screening) (**Appendix 1**).

Plates have been seeded with r-ALK 6 and 7 proteins both in their P and NON-P form, purified with both SEC and IEX. Seeding, with the different crystallization kits, has been repeated considering the following different conditions:

- different purified protein concentrations (3-5-7-10 mg/mL) to test saturation in presence of a specific precipitant agent;
- protein in complex with the PF-2341066 inhibitor;
- protein in complex with AMP-PNP inhibitor, an ATP analog;
- protein with and without DTT addition in the final buffer to avoid or facilitate disulfide bonds formation;
- presence of different precipitant solutions: protein ratios in the drops (1:1 or 1:2), because the necessary amount of salt, buffer and precipitant in each condition is related to the amount of protein and must be detected;
- plates stored at both 20 degrees and 4 degrees.

Unfortunately no crystal suitable for x-ray crystallography have been obtained yet in any of the tested conditions.

STD-NMR

STD-NMR (Saturation Transfer Difference–Nuclear Magnetic Resonance) is a recent technique used to characterize ligand-receptor complexes. NMR has been used for years for ligands screening in drug discovery, for the detection of intermolecular interactions. Understanding the binding process at a molecular level can help to identify new bioactive compounds.

This method is based on the transfer of magnetization from the protein to the bound ligands. The ligand is added to the protein in solution, protein is selectively irradiated, ligands that are in exchange between the bound and the free state become saturated. Saturated ligands are detected in solution. STD-NMR spectrum of molecules that bind to the protein is obtained by subtraction of a spectrum in which the protein is saturated (on-resonance) from one without protein saturation (off-resonance) (**Fig 55**) (Mayer, Meyer et al. 2011).

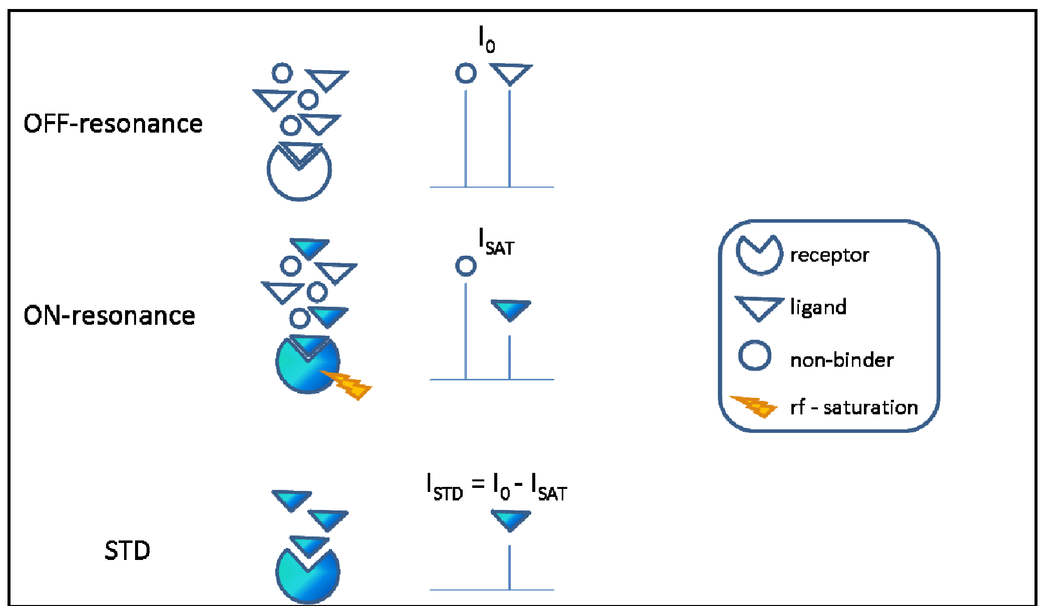


Fig 55_ STD-NMR.

STD-NMR spectrum (I_{STD}) is obtained by subtraction of the on-resonance spectrum (I_{SAT}) from the off-resonance spectrum (I_0).

The ALK 6 protein has been chosen for the NMR studies because of its already demonstrated stability and activity. The protein has been purified in order to obtain the NON-P form and concentrated. In this case, the P form has not been considered yet, to avoid the free ATP presence in the final solution. In fact, the ATP

could interfere with the ligand binding to the protein if, as we expect, the compound binds inside the ATP pocket.

The STD-NMR spectra are shown in **Figure 56**. The final STD-spectrum (spectrum 3) was obtained considering spectrum 1 as reference. Spectrum 2, produced by ligand irradiation in absence of the protein, represents the negative control of the experiment. The peaks in spectrum 3 correspond to ligand protons directly involved in the interaction between R458 and ALK (**Fig 56**).

In conclusion, the STD spectrum 3 demonstrated the binding between the compound and the protein. In particular, protons in position 5, 21 and 23, belonging to the aromatic portion of R458, revealed a stronger interaction with ALK (**Fig 56**). In fact, considering spectrum 1 and 3, the peaks corresponding to protons 5, 21 and 23 display a lower difference of intensity compared to all the other peaks (**Fig 56**). For resonance assignment see **Figure 57**. This is demonstrated by the fact that, in the spectrum, some peaks (corresponding to specific protons) show higher intensities than others.

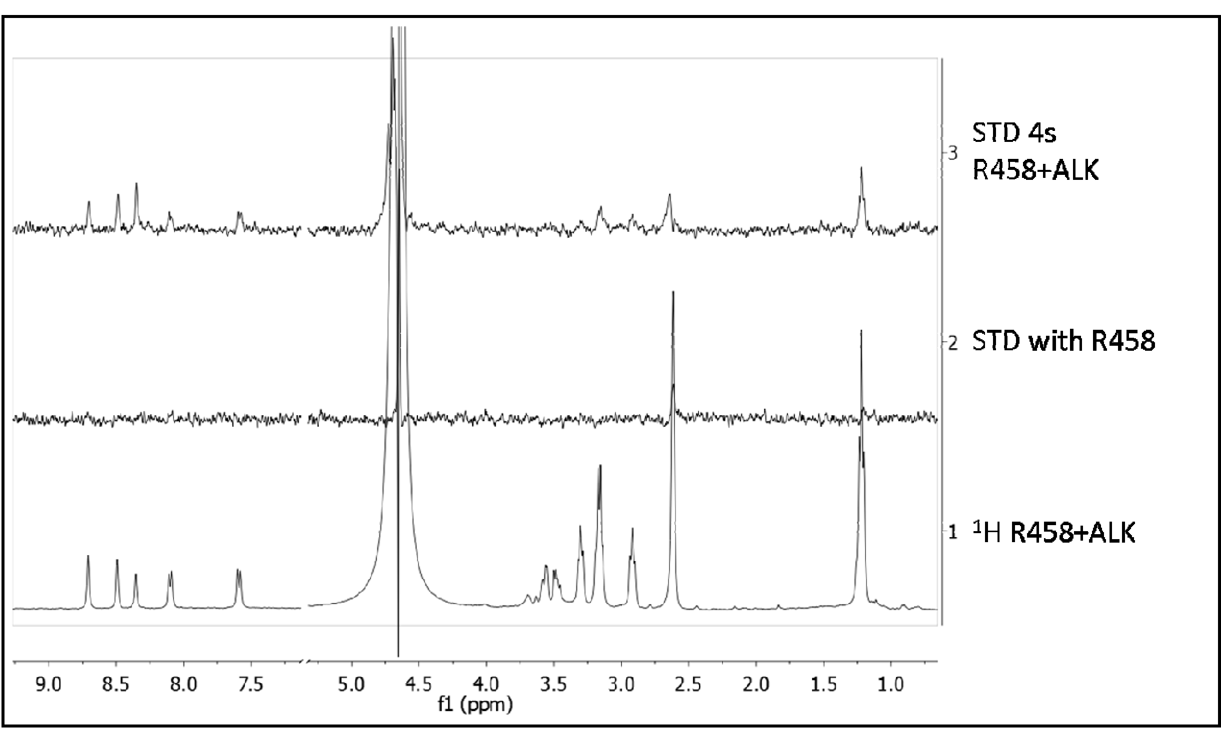


Fig 56_ STD-NMR result.

ALK 6 STD-NMR spectra results. In x-axis is the chemical shift (ppm), in y-axis is the absorbance. spectrum 1= reference proton of the mix (R458+ALK); spectrum 2 = only R458 in solution; spectrum 3 = STD R458+ALK result.

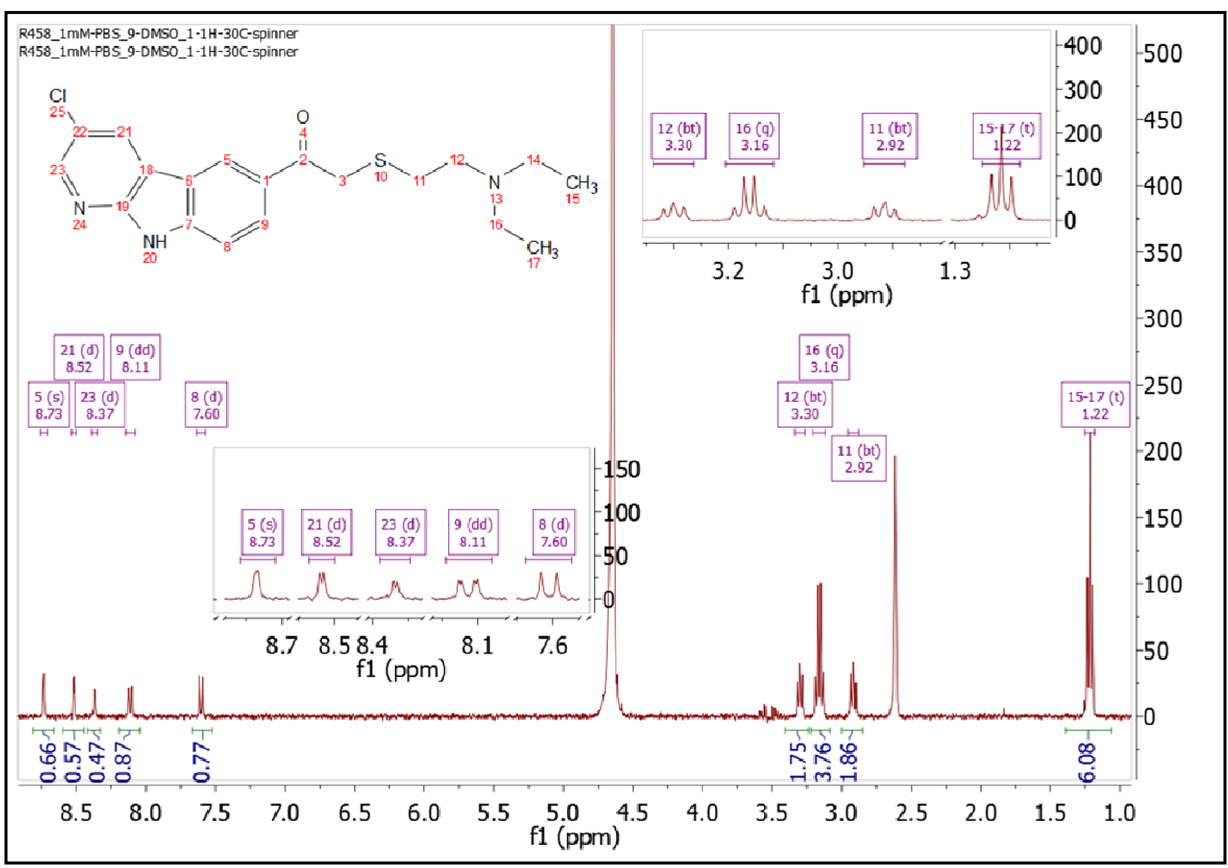


Fig 57_ R458 structure and STD-NMR.

Reference spectrum showing protons of R458 corresponding to each spectrum peak. The first 3 peaks from the left correspond to protons in position 5, 21 and 23 respectively.

The STD-NMR result confirms the R458-ALK interaction hypothesized by molecular modeling studies. Accordind to the molecular modeling, C in position 23 (marked in red), which revealed a strong interaction with the protein in STD test, is localized inside the ATP binding pocket near the hinge region backbone (**Fig 58**). The compound aromatic portion containing the 3 protons (in position 5, 21 and 23) showing ALK binding by STD, are placed toward the ATP binding pocket, while the compound chain goes outside the pocket (**Fig 58**).

Studies of competion with ATP by STD-NMR are ongoing to conferm the R458 binding inside the ATP pocket.

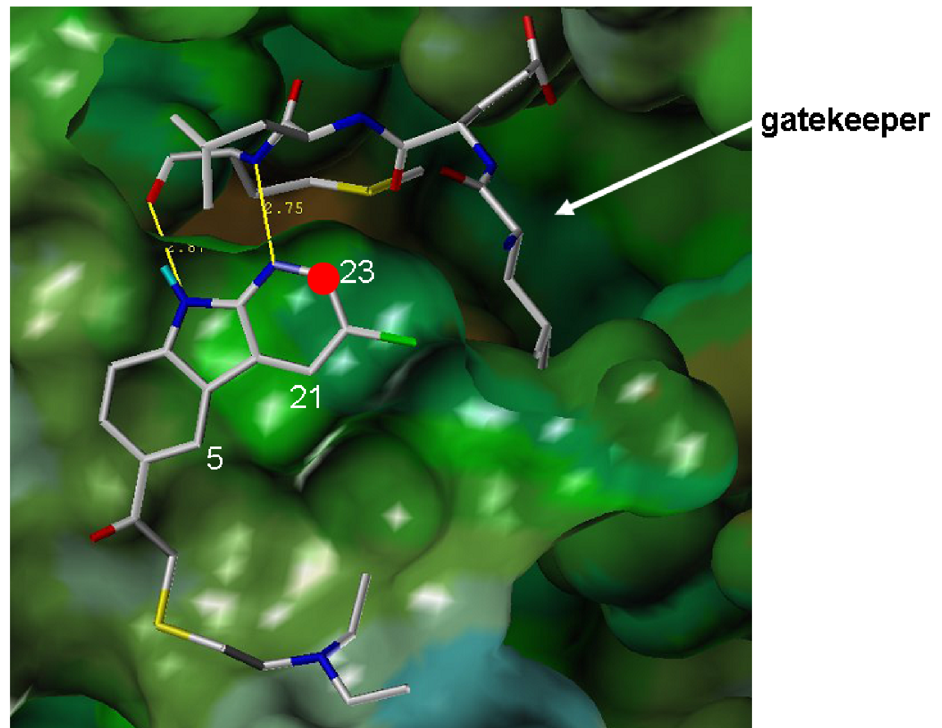


Fig 58_ Modeling of R458-ALK binding.

Result of molecular modeling showing R458-ALK binding. R458 and the backbone of the ALK hinge region are shown as colour coded stick models. Nitrogen atoms are shown in blue, oxygen atoms in red, sulphur atoms in yellow and chlorine atoms in green. Positions of the carbon atoms involved in the binding are in white. The protein surface is shown in green. Hydrogen bonds are represented by yellow lines.

DISCUSSION

Considering the number of both non-hematopoietic and hematopoietic diseases involving the ALK oncogenic tyrosine kinase, the need of a specific targeted therapy becomes evident. Our attention must be focused on the lack of an approved anticancer agent for ALCL treatment, especially in presence of tyrosine kinase mutations that often appears as a consequence of long-term cancer treatment (Choi, Soda et al. 2010 ; Sasaki, Okuda et al. 2010).

Structural characterization of the ALK kinase domain is fundamental to develop targeted therapy. In particular, information about the drug-protein interaction and about the inhibitors way of action can help to improve the small molecule efficiency. In the treatment of diseases caused by tyrosine kinases alterations, Imatinib, a Bcr/Abl inhibitor, development represents a good example to be followed. In fact, Imatinib is now used as current therapy in clinique for chronic myeloid leukemia (CML) treatment (Druker, Talpaz et al. 2001).

In order to study the structure of the ALK kinase domain, different constructs expressing the recombinant ALK kinase domain have been produced. Two different Baculovirus expression systems have been used and purification methods have been optimized to increase the yield and the purity of the r-proteins.

At the end two proteins, ALK 6 and 7, have been found to be suitable for structural studies (**Tab 1**). These proteins have been produced according to the BacPAK expression system. This system, together with an in batch purification protocol followed by chromatographic passages, has been demonstrated to be the best one concerning the amount of produced protein and the final sample purity. ALK 6 and 7 have a GST-tag completely removable by the proteolytic cleavage process and, as the GST-3C protease, it can be easily separated by the ALK protein thanks to the beads presence during the cleavage. Both phosphorylated and non-phosphorylated forms of the purified proteins have been studied. The final purified r-proteins are stable, in fact a concentration of 10 mg/mL can be reach without problem of protein precipitation; the preparation is very pure as seen in Coomassie gels; proteins are active, as displayed by the cold kinase assay and by the phosphorylation analysis, implying a right secondary structure conformation; finally the purified samples are homogeneous in terms of phosphorylation, as seen by IEX chromatography, and the aggregated fraction can be separated by SEC.

Considering the importance of tyrosine kinases mutation in drug-resistance appearance, also an ALK 6 mutant form, L1196M, has been produced for structural

characterization. L1196M mutation is related to the PF-2341066 inhibitor resistance in NSLC patients. We were able to obtain ALK 6 L1196M protein suitable for structural studies, both in the phosphorylated and non-phosphorylated forms thanks to the addition of the NVP-TAE684 inhibitor in cell culture. In particular, we observed a correlation between mutation and protein activity, in fact the mutant purified protein showed an intrinsic phosphorylation in contrary to the WT one.

The purified r-ALK WT and mutant forms (ALK 6 and ALK 6 L1196M) have been used to perform ELISA kinase assay to test their activity in presence of different ALK inhibitors. In this way we were able to test the inhibitory effect of specific compounds, in particular new drugs (R500B and R458) capacity to block the protein kinase activity has been validated.

To study the ALK kinase domain 3D structure, ALK 6 and 7 in their P and NON-P forms have been used for crystallization attempts. The study of both P (active) and NON-P (inactive) forms can give information about kinase activation. The PF-2341066 has been added to the purified protein to study the interaction between this compound and the protein, about 10000 different conditions for each protein have been tested for crystal formation without satisfying results.

ALK 6 has also been used for STD-NMR studies. The interaction between the kinase and R458, an azacarbazole compound studied in our laboratory as a new ALK inhibitor, has been analyzed. STD-NMR has revealed a binding between the aromatic portion of the molecule and the protein.

In conclusion, we purified and characterized different forms of the r-ALK kinase domain, including one mutant form, obtaining three forms suitable for structural studies. In particular, the purified proteins allowed the screening of conditions for crystallization, and the demonstration of an interaction between ALK kinase domain and a new kinase inhibitor by STD-NMR. In addition, the r-ALK protein has been used for screening of potential ALK inhibitors in ELISA kinase assay.

RECOMBINANT PROTEIN	EXPRESSION SYSTEM	CONSTRUCT	SEQUENCE	RESULT
ALK 1	Bac-to-Bac	GST-TEV	1099-1397	not suitable for structural study: not stable, not pure
ALK 2	Bac-to-Bac	GST-TEV	1099-1410	not suitable for structural study: not stable, not pure
ALK 3	Bac-to-Bac	GST-TEV	1099-1435	not suitable for structural study: not cleavable
ALK 4	Bac-to-Bac	GST-TEV	1099-1620	not suitable for structural study: not stable, low expression
ALK 5	BacPAK	GST-3C	1099-1620	not suitable for structural study: not stable
ALK 6	BacPAK	GST-3C	1064-1427	suitable for structural study
ALK 7	BacPAK	GST-3C	1064-1620	suitable for structural study
ALK 6 L1196M	BacPAK	GST-3C	1064-1427	suitable for structural study

Tab 1_ Summary of produced r-ALK proteins.

APPENDIX 1

Scheme of the 96-well sitting drop crystallization plate indicating wells position, and tables displaying the composition of the different crystallization kits.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

$AmSo_4$ Suite

Well	Salt	Buffer	Precipitant
A1			2.2 M Ammonium sulfate
A2	0.2 M Ammonium acetate		2.2 M Ammonium sulfate
A3	0.2 M Ammonium chloride		2.2 M Ammonium sulfate
A4	0.2 M Ammonium phosphate		2.2 M Ammonium sulfate
A5	0.2 M Ammonium fluoride		2.2 M Ammonium sulfate
A6	0.2 M Ammonium formate		2.2 M Ammonium sulfate
A7	0.18 M tri-Ammonium citrate		2.2 M Ammonium sulfate
A8	0.2 M di-Ammonium phosphate		2.2 M Ammonium sulfate
A9	0.2 M Ammonium iodide		2.2 M Ammonium sulfate
A10	0.2 M Ammonium nitrate		2.2 M Ammonium sulfate
A11	0.2 M di-Ammonium tartrate		2.2 M Ammonium sulfate
A12	0.2 M Cadmium chloride		2.2 M Ammonium sulfate
B1	0.2 M Cadmium sulfate		2.2 M Ammonium sulfate
B2	0.2 M Cesium chloride		2.2 M Ammonium sulfate
B3	0.2 M Cesium sulfate		2.2 M Ammonium sulfate
B4	0.2 M Ammonium bromide		2.2 M Ammonium sulfate
B5	0.2 M Lithium acetate		2.2 M Ammonium sulfate
B6	0.2 M Lithium chloride		2.2 M Ammonium sulfate
B7	0.2 M tri-Lithium citrate		2.2 M Ammonium sulfate
B8	0.2 M Lithium nitrate		2.2 M Ammonium sulfate
B9	0.2 M Lithium sulfate		2.2 M Ammonium sulfate
B10	0.2 M Potassium acetate		2.2 M Ammonium sulfate
B11	0.2 M Potassium bromide		2.2 M Ammonium sulfate
B12	0.2 M Potassium chloride		2.2 M Ammonium sulfate
C1	0.2 M tri-Potassium citrate		2.2 M Ammonium sulfate
C2	0.2 M Potassium phosphate		2.2 M Ammonium sulfate
C3	0.2 M Potassium fluoride		2.2 M Ammonium sulfate

C4	0.2 M Potassium formate		2.2 M Ammonium sulfate
C5	0.2 M di-Potassium phosphate		2.2 M Ammonium sulfate
C6	0.2 M Potassium iodide		2.2 M Ammonium sulfate
C7	0.2 M Potassium nitrate		2.2 M Ammonium sulfate
C8	0.2 M K/Na tartrate		2.2 M Ammonium sulfate
C9	0.2 M Potassium sulfate		2.2 M Ammonium sulfate
C10	0.2 M Potassium thiocyanate		2.2 M Ammonium sulfate
C11	0.2 M Sodium acetate		2.2 M Ammonium sulfate
C12	0.2 M Sodium bromide		2.2 M Ammonium sulfate
D1	0.2 M Sodium chloride		2.2 M Ammonium sulfate
D2	0.2 M tri-Sodium citrate		2.2 M Ammonium sulfate
D3	0.2 M Sodium phosphate		2.2 M Ammonium sulfate
D4	0.2 M Sodium fluoride		2.2 M Ammonium sulfate
D5	0.2 M Sodium formate		2.2 M Ammonium sulfate
D6	0.2 M di-Sodium phosphate		2.2 M Ammonium sulfate
D7	0.2 M Sodium iodide		2.2 M Ammonium sulfate
D8	0.2 M Sodium malonate		2.2 M Ammonium sulfate
D9	0.2 M Sodium nitrate		2.2 M Ammonium sulfate
D10	0.2 M Sodium sulfate		2.2 M Ammonium sulfate
D11	0.2 M di-Sodium tartate		2.2 M Ammonium sulfate
D12	0.2 M Sodium thiocyanate		2.2 M Ammonium sulfate
E1		0.1 M Citric acid pH 4.0	0.8 M Ammonium sulfate
E2		0.1 M Citric acid pH 5.0	0.8 M Ammonium sulfate
E3		0.1 M MES pH 6.0	0.8 M Ammonium sulfate
E4		0.1 M HEPES pH 7.0	0.8 M Ammonium sulfate
E5		0.1 M TRIS pH 8.0	0.8 M Ammonium sulfate
E6		0.1 M BICINE pH 9.0	0.8 M Ammonium sulfate
E7		0.1 M Citric acid pH 4.0	1.6 M Ammonium sulfate
E8		0.1 M Citric acid pH 5.0	1.6 M Ammonium sulfate
E9		0.1 M MES pH 6.0	1.6 M Ammonium sulfate
E10		0.1 M HEPES pH 7.0	1.6 M Ammonium sulfate
E11		0.1 M TRIS pH 8.0	1.6 M Ammonium sulfate
E12		0.1 M BICINE pH 9.0	1.6 M Ammonium sulfate
F1		0.1 M Citric acid pH 4.0	2.4 M Ammonium sulfate
F2		0.1 M Citric acid pH 5.0	2.4 M Ammonium sulfate
F3		0.1 M MES pH 6.0	2.4 M Ammonium sulfate
F4		0.1 M HEPES pH 7.0	2.4 M Ammonium sulfate
F5		0.1 M TRIS pH 8.0	2.4 M Ammonium sulfate
F6		0.1 M BICINE pH 9.0	2.4 M Ammonium sulfate
F7		0.1 M Citric acid pH 4.0	3.2 M Ammonium sulfate
F8		0.1 M Citric acid pH 5.0	3.2 M Ammonium sulfate
F9		0.1 M MES pH 6.0	3.2 M Ammonium sulfate
F10		0.1 M HEPES pH 7.0	3.2 M Ammonium sulfate
F11		0.1 M TRIS pH 8.0	3.2 M Ammonium sulfate
F12		0.1 M BICINE pH 9.0	3.2 M Ammonium sulfate
G1	0.1 M tri-Sodium citrate		0.5 M Ammonium sulfate, 1.0 M Lithium Sulfate
G2			1.0 M Ammonium sulfate
G3		0.1 M Sodium acetate pH 4.6	1.0 M Ammonium sulfate
G4		0.1 M HEPES sodium salt pH 7.5	1.0 M Ammonium sulfate, 2 %(w/v) PEG 400
G5		0.1 M TRIS.HCl pH 8.5	1.0 M Ammonium sulfate
G6	0.05 M tri-Sodium citrate		1.2 M Ammonium sulfate, 3 %(w/v) Isopropanol
G7		0.1 M TRIS.HCl pH 8.5	1.5 M Ammonium sulfate, 15 %(w/v) Glycerol

G8	0.5 M Lithium chloride		1.6 M Ammonium sulfate
G9	1.0 M Lithium sulfate		1.6 M Ammonium sulfate
G10	0.2 M Sodium chloride	0.1 M HEPES sodium salt pH 7.5	1.6 M Ammonium sulfate
G11		0.1 M HEPES sodium salt pH 7.5	1.6 M Ammonium sulfate, 2 %(w/v) PEG 1000
G12		0.1 M MES sodium salt pH 6.5	1.8 M Ammonium sulfate
H1	2.0 M Sodium chloride		2.0 M Ammonium sulfate
H2		0.1 M Sodium acetate pH 4.6	2.0 M Ammonium sulfate
H3		0.1 M MES sodium salt pH 6.5	2.0 M Ammonium sulfate, 5 %(w/v) PEG 400
H4		0.1 M TRIS.HCl pH 8.5	2.0 M Ammonium sulfate
H5			2.2 M Ammonium sulfate
H6			2.2 M Ammonium sulfate, 20 %(w/v) Glycerol
H7	0.1 M tri-Sodium citrate		2.4 M Ammonium sulfate
H8			3.0 M Ammonium sulfate, 1 %(w/v) MPD
H9			3.0 M Ammonium sulfate, 10 %(w/v) Glycerol
H10		0.1 M HEPES sodium salt pH 7.5	3.5 M Ammonium sulfate
H11		0.1 M MES sodium salt pH 6.5	3.5 M Ammonium sulfate, 1 %(w/v) MPD
H12			3.5 M Ammonium sulfate

Classics Lite Suite

Well	Salt	Buffer	Precipitant
A1	0.01 M Cobalt chloride	0.1 M Sodium acetate pH 4.6	0.5 M 1,6-Hexanediol
A2		0.1 M tri-Sodium citrate pH 5.6	1.25 M 1,6-Hexanediol
A3	0.2 M Magnesium chloride	0.1 M TRIS pH 8.5	1.7 M 1,6-Hexanediol
A4			2.5 %(v/v) Isopropanol, 1.0 M Ammonium sulfate
A5		0.1 M HEPES sodium salt pH 7.5	5 %(v/v) Isopropanol, 10 %(w/v) PEG 4000
A6	0.2 M Calcium chloride	0.1 M Sodium acetate pH 4.6	10 %(v/v) Isopropanol
A7		0.1 M tri-Sodium citrate pH 5.6	10 %(v/v) Isopropanol, 10 %(w/v) PEG 4000
A8	0.2 M tri-Sodium citrate	0.1 M HEPES sodium salt pH 7.5	10 %(v/v) Isopropanol
A9	0.2 M tri-Sodium citrate	0.1 M Sodium cacodylate pH 6.5	15 %(v/v) Isopropanol
A10	0.2 M Magnesium chloride	0.1 M HEPES sodium salt pH 7.5	15 %(v/v) Isopropanol
A11	0.2 M Ammonium acetate	0.1 M TRIS.HCl pH 8.5	15 %(v/v) Isopropanol
A12			5 %(v/v) Ethanol, 0.75 M Sodium chloride
B1		0.1 M TRIS pH 8.5	10 %(v/v) Ethanol
B2			12.5 %(v/v) Ethylene glycol
B3	0.02 M Calcium chloride	0.1 M Sodium acetate pH 4.6	15 %(v/v) MPD
B4	0.2 M Sodium chloride	0.1 M Sodium acetate pH 4.6	15 %(v/v) MPD
B5	0.2 M Ammonium acetate	0.1 M tri-Sodium citrate pH 5.6	15 %(v/v) MPD
B6	0.2 M Magnesium acetate	0.1 M Sodium cacodylate pH 6.5	15 %(v/v) MPD
B7	0.2 M tri-Sodium citrate	0.1 M HEPES sodium salt pH 7.5	15 %(v/v) MPD
B8	0.5 M Ammonium sulfate	0.1 M HEPES pH 7.5	15 %(v/v) MPD
B9	0.2 M Ammonium phosphate	0.1 M TRIS pH 8.5	25 %(v/v) MPD
B10		0.1 M HEPES pH 7.5	35 %(v/v) MPD
B11		0.1 M TRIS pH 8.5	12.5 %(v/v) tert-Butanol
B12		0.1 M tri-Sodium citrate pH 5.6	17.5 %(v/v) tert-Butanol
C1			0.2 M Ammonium phosphate
C2		0.1 M tri-Sodium citrate pH 5.6	0.5 M Ammonium phosphate
C3		0.1 M TRIS.HCl pH 8.5	1.0 M Ammonium phosphate
C4		0.1 M HEPES pH 7.5	1.0 M Ammonium formate
C5		0.1 M Sodium acetate pH 4.6	1.0 M Ammonium sulfate
C6		0.1 M TRIS.HCl pH 8.5	1.0 M Ammonium sulfate
C7			1.0 M Ammonium sulfate
C8	0.1 M Sodium chloride	0.1 M HEPES pH 7.5	0.8 M Ammonium sulfate

C9	0.01 M Cobalt chloride	0.1 M MES pH 6.5	0.9 M Ammonium sulfate
C10	0.2 M K/Na tartrate	0.1 M tri-Sodium citrate pH 5.6	1 M Ammonium sulfate
C11			0.5 M Imidazole pH 7.0
C12			0.2 M K/Na tartrate
D1		0.1 M HEPES sodium salt pH 7.5	0.4 M K/Na tartrate
D2		0.1 M Imidazole pH 6.5	0.5 M Sodium acetate
D3	0.05 M Cadmium sulfate	0.1 M HEPES pH 7.5	0.5 M Sodium acetate
D4		0.1 M Sodium cacodylate pH 6.5	0.7 M Sodium acetate
D5		0.1 M Sodium acetate pH 4.6	1.0 M Sodium chloride
D6	0.1 M Sodium phosphate, 0.1 M Potassium phosphate	0.1 M MES pH 6.5	1.0 M Sodium chloride
D7		0.1 M HEPES pH 7.5	2.15 M Sodium chloride
D8		0.1 M HEPES sodium salt pH 7.5	0.7 M tri-Sodium citrate
D9			0.8 M tri-Sodium citrate pH 6.5
D10		0.1 M HEPES sodium salt pH 7.5	0.4 M Sodium phosphate, 0.4 M Potassium phosphate
D11		0.1 M Sodium acetate pH 4.6	1.0 M Sodium formate
D12			2.0 M Sodium formate
E1		0.1 M BICINE pH 9.0	1 %(v/v) Dioxane, 5 %(w/v) PEG 20000
E2		0.1 M MES pH 6.5	5 %(v/v) Dioxane, 0.8 M Ammonium sulfate
E3			17.5 %(v/v) Dioxane
E4	0.5 M Sodium chloride	0.1 M tri-Sodium citrate pH 5.6	1 %(v/v) Ethylene imine polymer
E5		0.1 M TRIS pH 8.5	6 %(v/v) Glycerol, 0.75 M Ammonium sulfate
E6	0.25 M Sodium chloride, 0.005 M Magnesium chloride		0.01 M CTAB
E7	0.01 M Ferric chloride	0.1 M tri-Sodium citrate pH 5.6	5 %(v/v) Jeffamine M-600
E8		0.1 M HEPES pH 7.5	10 %(v/v) Jeffamine M-600
E9	0.5 M Ammonium sulfate	0.1 M tri-Sodium citrate pH 5.6	0.5 M Lithium sulfate
E10	0.01 M Nickel chloride	0.1 M TRIS pH 8.5	0.5 M Lithium sulfate
E11		0.1 M HEPES sodium salt pH 7.5	0.75 M Lithium sulfate
E12		0.1 M BICINE pH 9.0	1.0 M Magnesium chloride
F1			0.1 M Magnesium formate
F2		0.1 M MES pH 6.5	0.8 M Magnesium sulfate
F3		0.1 M TRIS.HCl pH 8.5	4 %(w/v) PEG 8000
F4		0.1 M HEPES pH 7.5	5 %(w/v) PEG 8000
F5	0.5 M Lithium sulfate		7.5 %(w/v) PEG 8000
F6	0.2 M Zinc acetate	0.1 M Sodium cacodylate pH 6.5	9 %(w/v) PEG 8000
F7	0.2 M Calcium acetate	0.1 M Sodium cacodylate pH 6.5	9 %(w/v) PEG 8000
F8	0.2 M Magnesium acetate	0.1 M Sodium cacodylate pH 6.5	10 %(w/v) PEG 8000
F9	0.05 M Potassium phosphate		10 %(w/v) PEG 8000
F10	0.2 M Ammonium sulfate	0.1 M Sodium cacodylate pH 6.5	15 %(w/v) PEG 8000
F11	0.2 M Sodium acetate	0.1 M Sodium cacodylate pH 6.5	15 %(w/v) PEG 8000
F12	0.2 M Ammonium sulfate		15 %(w/v) PEG 8000
G1		0.1 M HEPES sodium salt pH 7.5	1 %(v/v) PEG 400, 1.0 M Ammonium sulfate
G2	0.2 M Calcium chloride	0.1 M HEPES sodium salt pH 7.5	14 %(v/v) PEG 400
G3	0.1 M Cadmium chloride	0.1 M Sodium acetate pH 4.6	15 %(v/v) PEG 400
G4	0.2 M Magnesium chloride	0.1 M HEPES sodium salt pH 7.5	15 %(v/v) PEG 400
G5	0.2 M tri-Sodium citrate	0.1 M TRIS.HCl pH 8.5	15 %(v/v) PEG 400
G6	0.1 M Sodium chloride	0.1 M BICINE pH 9.0	10 %(w/v) PEG 550 MME
G7	0.01 M Zinc sulfate	0.1 M MES pH 6.5	12.5 %(w/v) PEG 550 MME
G8			5 %(w/v) PEG 1000, 5 %(w/v) PEG 8000
G9			15 %(w/v) PEG 1500
G10	0.01 M Nickel chloride	0.1 M TRIS pH 8.5	10 %(w/v) PEG 2000 MME
G11	0.2 M Ammonium sulfate	0.1 M Sodium acetate pH 4.6	15 %(w/v) PEG 2000 MME

G12		0.1 M Sodium acetate pH 4.6	4 %(w/v) PEG 4000
H1	0.2 M Ammonium sulfate	0.1 M Sodium acetate pH 4.6	12.5 %(w/v) PEG 4000
H2	0.2 M Ammonium acetate	0.1 M Sodium acetate pH 4.6	15 %(w/v) PEG 4000
H3	0.2 M Ammonium acetate	0.1 M tri-Sodium citrate pH 5.6	15 %(w/v) PEG 4000
H4	0.2 M Magnesium chloride	0.1 M TRIS.HCl pH 8.5	15 %(w/v) PEG 4000
H5	0.2 M Lithium sulfate	0.1 M TRIS.HCl pH 8.5	15 %(w/v) PEG 4000
H6	0.2 M Sodium acetate	0.1 M TRIS.HCl pH 8.5	15 %(w/v) PEG 4000
H7	0.2 M Ammonium sulfate		15 %(w/v) PEG 4000
H8	0.2 M Ammonium sulfate	0.1 M MES pH 6.5	15 %(w/v) PEG 5000 MME
H9		0.1 M HEPES pH 7.5	5 %(w/v) PEG 6000, 2.5 %(v/v) MPD
H10			5 %(w/v) PEG 6000, 1.0 M Sodium chloride
H11		0.1 M HEPES pH 7.5	10 %(w/v) PEG 10000, 4 %(v/v) Ethylene glycol
H12		0.1 M MES pH 6.5	6 %(w/v) PEG 20000

JCSG Core Suite IV

Well	Salt	Buffer	Precipitant	Final pH
A01	0.2 M Lithium sulfate	0.1 M CAPS pH 10.5	2.0 M Ammonium sulfate	
A02	0.2 M Lithium sulfate	0.1 M Glycine pH 10.5	1.2 M Sodium dihydrogen phosphate/0.8 M di-Potassium hydrogen phosphate	
A03		0.1M CAPS pH 10.5	40%(v/v) MPD	
A04		0.1 M CHES pH 9.5	10%(w/v) PEG 3000	
A05	0.2 M Lithium sulfate	0.1 M CHES pH 9.5	1.0 M Sodium/Potassium tartrate	
A06		0.1 M CHES pH 9.5	30%(v/v) PEG 400	
A07		0.1 M CHES pH 9.5	15%(v/v) Ethanol	
A08	0.2 M Sodium citrate	0.1M CHES pH 9.5	40%(v/v) PEG 300	
A09		0.1M CHES pH 9.5	40%(v/v) MPD	
A10		0.1 M Bicine pH 9.0	1.6 M Ammonium sulfate	9,0
A11		0.1 M Bicine pH 9.0	0.8 M Ammonium sulfate	9,0
A12		0.1 M Bicine pH 9.0	2.4 M Ammonium sulfate	9,0
B01		0.1 M Bicine pH 8.5	10%(w/v) PEG 6000	9,0
B02		0.1 M Bicine pH 9.0	2.4 M Ammonium sulfate	
B03		0.1 M Bicine pH 8.5	30%(w/v) PEG 6000	9,0
B04		0.1 M Bicine pH 8.5	65%(v/v) MPD	9,0
B05		0.1 M Bicine pH 9.0	2.0 M Magnesium chloride	
B06		0.1 M Tris pH 8.5	10%(v/v) Isopropanol	
B07	0.2 M Magnesium chloride	0.1M Tris pH 8.5	50%(v/v) Ethylene glycol	
B08	0.2 M Magnesium chloride	0.1M Tris pH 8.5	25%(v/v) 1,2-Propanediol, 10%(v/v) Glycerol	
B09	0.2 M Magnesium chloride	0.1 M Tris·HCl pH 8.5	30%(w/v) PEG 4000	
B10	0.2 M Sodium citrate	0.1 M Tris·HCl pH 8.5	30%(v/v) PEG 400	
B11	0.2 M Lithium sulfate	0.1 M Tris·HCl pH 8.5	30%(w/v) PEG 4000	
B12	0.2 M Ammonium acetate	0.1 M Tris-HCl pH 8.5	30%(v/v) Isopropanol	
C01		0.1 M Tris pH 8.5	1.5 M Ammonium sulfate, 12%(v/v) Glycerol	
C02	0.18 M tri-Sodium citrate	0.09 M TRIS.HCl pH 8.5	27 %(v/v) PEG 400, 10 %(v/v) Glycerol	
C03	0.17 M Sodium acetate	0.085 M Tris·HCl pH 8.5	25.5%(w/v) PEG 4000, 15%(v/v) Glycerol	
C04		0.1 M Imidazole pH 8.0	10%(v/v) Isopropanol	
C05	0.2 M Zinc acetate	0.1 M Imidazole pH 8.0	2.5 M Sodium chloride	
C06		0.1 M Imidazole pH 8.0	2.5 M Sodium chloride	
C07		0.1 M Imidazole pH 8.0	10%(w/v) PEG 8000	
C08	0.2 M Sodium chloride	0.1 M Imidazole pH 8.0	1.0 M di-Ammonium phosphate	
C09		0.1 M Tris pH 8.5	1.6 M Ammonium sulfate	8,0

C10		0.1 M Tris pH 8.5	5%(w/v) PEG 6000	8,0
C11		0.1 M Tris pH 8.5	65%(v/v) MPD	8,0
C12	1.0 M Lithium chloride	0.1 M Tris pH 8.5	10%(w/v) PEG 6000	8,0
D01		0.1 M Tris pH 8.0	3.2 M Ammonium sulfate	
D02		0.1 M HEPES pH 7.5	1.26 M Ammonium sulfate	
D03	0.2 M Sodium chloride	0.1 M HEPES pH 7.5	35%(v/v) MPD	
D04		0.1M HEPES pH 7.5	50%(v/v) PEG 200	
D05		0.1 M HEPES pH 7.5	1.5 M Lithium sulfate	
D06		0.1 M HEPES pH 7.5	4.3 M Sodium chloride	
D07	0.2 M Sodium citrate	0.1 M HEPES pH 7.5	30%(v/v) MPD	
D08		0.1 M HEPES pH 7.5	20%(w/v) PEG 10000, 8%(v/v) Ethylene glycol	
D09		0.09 M HEPES pH 7.5	1.26 M tri-Sodium citrate, 10%(v/v) Glycerol	
D10	1.7 M Ammonium sulfate	0.085 M HEPES pH 7.5	1.7%(v/v) PEG 400, 15%(v/v) Glycerol	
D11	0.05 M Lithium sulfate	0.1M HEPES pH 7.5	30%(v/v) PEG 600,10%(v/v) Glycerol	
D12		0.1M HEPES pH 7.5	30%(v/v) 1,2-Propanediol, 20%(v/v) PEG 400	
E01	0.2 M Ammonium sulfate	0.1M Tris pH 7.0	25%(v/v) 1,2-Propanediol, 10%(v/v) Glycerol	
E02		0.1M HEPES pH 7.5	5%(w/v) PEG 3000, 40%(v/v) Ethylene glycol	
E03	0.2 M Ammonium sulfate	0.1M Tris pH 7.0	40%(v/v) MPD	
E04			4.0 M Sodium formate	
E05			3.6 M Sodium formate, 10%(v/v) Glycerol	
E06	0.2 M Calcium acetate	0.1M HEPES pH 7.5	40%(v/v) PEG 400	
E07	0.2 M Sodium chloride	0.1 M Tris pH 7.0	30%(w/v) PEG 3000	
E08	0.2 M Lithium sulfate	0.1 M Tris pH 7.0	1.0 M Sodium/Potassium tartrate	
E09	0.2 M Calcium acetate	0.1M Sodium cacodylate pH 6.5	40%(v/v) PEG 600	
E10		0.1 M HEPES pH 6.5	0.8 M Ammonium sulfate	7,0
E11		0.1 M HEPES pH 7.0	3.2 M Ammonium sulfate	
E12		0.1 M HEPES pH 6.5	30%(w/v) PEG 6000	7,0
F01	1.0 M Lithium chloride	0.1 M HEPES pH 7.0		
F02	1 M Sodium chloride	0.1M Sodium cacodylate pH 6.5	30%(v/v) PEG 600, 10%(v/v) Glycerol	
F03	0.2 M Zinc acetate	0.1 M Sodium cacodylate pH 6.5	10%(v/v) Isopropanol	
F04	0.2 M Calcium acetate	0.1M Sodium cacodylate pH 6.5	45%(v/v) Glycerol	
F05		0.1 M HEPES pH 7.0	30%(v/v) Jeffamine M-600	7,0
F06	0.1 M Sodium dihydrogen phosphate/ 0.1 M potassium dihydrogen phosphate	0.1 M MES pH 6.5	2.0 M Sodium chloride	
F07	0.16 M Zinc acetate	0.08 M Sodium cacodylate pH 6.5	14.4%(w/v) PEG 8000, 20%(v/v) Glycerol	
F08		0.1M Sodium citrate pH 5.5	30%(v/v) 1,2-Propanediol, 20%(v/v) MPD	
F09	0.2 M Zinc acetate		20%(w/v) PEG 3350	
F10		0.1M Sodium citrate pH 5.5	5%(w/v) PEG 1000, 35%(v/v) Isopropanol	
F11		0.1M MES pH 6.0	30%(v/v) PEG 600, 5%(w/v) PEG 1000, 10%(v/v) Glycerol	
F12		0.1M Sodium citrate pH 5.5	40%(v/v) MPD	
G01	0.2 M Zinc acetate	0.1M Imidazole pH 8.0	35%(v/v) Isopropanol	
G02		0.1 M MES pH 6.0	1.0 M Sodium/Potassium tartrate	
G03	0.2 M Lithium sulfate	0.1 M MES pH 6.0	20%(v/v) Butanediol	
G04	0.2 M Zinc acetate	0.1 M MES pH 6.0	15%(v/v) Ethanol	
G05		0.1 M MES pH 5.0	1.6 M Ammonium sulfate	6,0
G06		0.1 M MES pH 5.0	30%(w/v) PEG 6000	6,0
G07	0.2 M Zinc acetate	0.1M Imidazole pH 8.0	40%(v/v) PEG 300	

G08	0.2 M Ammonium acetate	0.1 M Sodium citrate pH 5.6	30%(v/v) MPD	
G09	0.01 M Iron(II)chloride	0.1 M Sodium citrate pH 5.6	10%(v/v) Jeffamine M-600	
G10	0.7 M Ammonium dihydrogen phosphate	0.07 M Sodium citrate pH 5.6	30%(v/v) Glycerol	
G11	0.2 M Lithium sulfate	0.1 M Sodium citrate pH 5.5	15%(v/v) Ethanol	
G12	0.05 M Calcium acetate	0.1M Sodium acetate pH 4.5	40%(v/v) 1,2-Propanediol	
H01		0.1M Sodium acetate pH 4.5	35%(v/v) Isopropanol	
H02	0.2 M Ammonium acetate	0.1 M Sodium acetate pH 4.6	30%(w/v) PEG 4000	
H03	0.17 M Ammonium acetate	0.085 M Sodium acetate pH 4.6	25.5%(w/v) PEG 4000, 15%(v/v) Glycerol	
H04	0.2 M Zinc acetate	0.1 M Sodium acetate pH 4.5	20%(w/v) PEG 1000	
H05		0.1 M Sodium acetate pH 4.5	1.0 M di-Ammonium phosphate	
H06		0.1 M Sodium acetate pH 4.5	0.8 M Sodium dihydrogen phosphate/1.2 M di-Potassium hydrogen phosphate	
H07	0.2 M Ammonium sulfate	0.1M Phosphate-citrate pH 4.2	40%(v/v) Ethylene glycol	
H08			10%(v/v) Ethanol, 1.5 M Sodium chloride	
H09			1.5 M Ammonium sulfate, 25%(v/v) Glycerol	
H10		0.1 M Phosphate-citrate pH 4.2	1.6 M Sodium dihydrogen phosphate/0.4 M di-Potassium hydrogen phosphate	
H11		0.1 M Citric Acid pH 2.5	30%(w/v) PEG 6000	4,0
H12	1.0 M Lithium chloride	0.1 M Citric Acid	30%(w/v) PEG 6000	4,0

PEGs Suite

Well	Salt	Buffer	Precipitant
A1		0.1 M Sodium acetate pH 4.6	40 %(v/v) PEG 200
A2		0.1 M Sodium acetate pH 4.6	30 %(v/v) PEG 300
A3		0.1 M Sodium acetate pH 4.6	30 %(v/v) PEG 400
A4		0.1 M Sodium acetate pH 4.6	25 %(v/v) PEG 550 MME
A5		0.1 M Sodium acetate pH 4.6	25 %(w/v) PEG 1000
A6		0.1 M Sodium acetate pH 4.6	25 %(w/v) PEG 2000 MME
A7		0.1 M MES pH 6.5	40 %(v/v) PEG 200
A8		0.1 M MES pH 6.5	30 %(v/v) PEG 300
A9		0.1 M MES pH 6.5	30 %(v/v) PEG 400
A10		0.1 M MES pH 6.5	25 %(v/v) PEG 550 MME
A11		0.1 M MES pH 6.5	25 %(w/v) PEG 1000
A12		0.1 M MES pH 6.5	25 %(w/v) PEG 2000 MME
B1		0.1 M Sodium HEPES pH 7.5	40 %(v/v) PEG 200
B2		0.1 M Sodium HEPES pH 7.5	30 %(v/v) PEG 300
B3		0.1 M Sodium HEPES pH 7.5	30 %(v/v) PEG 400
B4		0.1 M Sodium HEPES pH 7.5	25 %(v/v) PEG 550 MME
B5		0.1 M Sodium HEPES pH 7.5	25 %(w/v) PEG 1000
B6		0.1 M Sodium HEPES pH 7.5	25 %(w/v) PEG 2000 MME
B7		0.1 M TRIS.HCl pH 8.5	40 %(v/v) PEG 200
B8		0.1 M TRIS.HCl pH 8.5	30 %(v/v) PEG 300
B9		0.1 M TRIS.HCl pH 8.5	30 %(v/v) PEG 400
B10		0.1 M TRIS.HCl pH 8.5	25 %(v/v) PEG 550 MME
B11		0.1 M TRIS.HCl pH 8.5	25 %(w/v) PEG 1000
B12		0.1 M TRIS.HCl pH 8.5	25 %(w/v) PEG 2000 MME
C1		0.1 M Sodium acetate pH 4.6	25 %(w/v) PEG 3000
C2		0.1 M Sodium acetate pH 4.6	25 %(w/v) PEG 4000
C3		0.1 M Sodium acetate pH 4.6	25 %(w/v) PEG 6000
C4		0.1 M Sodium acetate pH 4.6	25 %(w/v) PEG 8000

C5		0.1 M Sodium acetate pH 4.6	20 %(w/v) PEG 10000
C6		0.1 M Sodium acetate pH 4.6	15 %(w/v) PEG 20000
C7		0.1 M MES pH 6.5	25 %(w/v) PEG 3000
C8		0.1 M MES pH 6.5	25 %(w/v) PEG 4000
C9		0.1 M MES pH 6.5	25 %(w/v) PEG 6000
C10		0.1 M MES pH 6.5	25 %(w/v) PEG 8000
C11		0.1 M MES pH 6.5	20 %(w/v) PEG 10000
C12		0.1 M MES pH 6.5	15 %(w/v) PEG 20000
D1		0.1 M Sodium HEPES pH 7.5	25 %(w/v) PEG 3000
D2		0.1 M Sodium HEPES pH 7.5	25 %(w/v) PEG 4000
D3		0.1 M Sodium HEPES pH 7.5	25 %(w/v) PEG 6000
D4		0.1 M Sodium HEPES pH 7.5	25 %(w/v) PEG 8000
D5		0.1 M Sodium HEPES pH 7.5	20 %(w/v) PEG 10000
D6		0.1 M Sodium HEPES pH 7.5	15 %(w/v) PEG 20000
D7		0.1 M TRIS.HCl pH 8.5	25 %(w/v) PEG 3000
D8		0.1 M TRIS.HCl pH 8.5	25 %(w/v) PEG 4000
D9		0.1 M TRIS.HCl pH 8.5	25 %(w/v) PEG 6000
D10		0.1 M TRIS.HCl pH 8.5	25 %(w/v) PEG 8000
D11		0.1 M TRIS.HCl pH 8.5	20 %(w/v) PEG 10000
D12		0.1 M TRIS.HCl pH 8.5	15 %(w/v) PEG 20000
E1	0.2 M Sodium fluoride		20 %(w/v) PEG 3350
E2	0.2 M Potassium fluoride		20 %(w/v) PEG 3350
E3	0.2 M Ammonium fluoride		20 %(w/v) PEG 3350
E4	0.2 M Lithium chloride		20 %(w/v) PEG 3350
E5	0.2 M Magnesium chloride		20 %(w/v) PEG 3350
E6	0.2 M Sodium chloride		20 %(w/v) PEG 3350
E7	0.2 M Calcium chloride		20 %(w/v) PEG 3350
E8	0.2 M Potassium chloride		20 %(w/v) PEG 3350
E9	0.2 M Ammonium chloride		20 %(w/v) PEG 3350
E10	0.2 M Sodium iodide		20 %(w/v) PEG 3350
E11	0.2 M Potassium iodide		20 %(w/v) PEG 3350
E12	0.2 M Ammonium iodide		20 %(w/v) PEG 3350
F1	0.2 M Sodium thiocyanate		20 %(w/v) PEG 3350
F2	0.2 M Potassium thiocyanate		20 %(w/v) PEG 3350
F3	0.2 M Lithium nitrate		20 %(w/v) PEG 3350
F4	0.2 M Magnesium nitrate		20 %(w/v) PEG 3350
F5	0.2 M Sodium nitrate		20 %(w/v) PEG 3350
F6	0.2 M Potassium nitrate		20 %(w/v) PEG 3350
F7	0.2 M Ammonium nitrate		20 %(w/v) PEG 3350
F8	0.2 M Magnesium formate		20 %(w/v) PEG 3350
F9	0.2 M Sodium formate		20 %(w/v) PEG 3350
F10	0.2 M Potassium formate		20 %(w/v) PEG 3350
F11	0.2 M Ammonium formate		20 %(w/v) PEG 3350
F12	0.2 M Lithium acetate		20 %(w/v) PEG 3350
G1	0.2 M Magnesium acetate		20 %(w/v) PEG 3350
G2	0.2 M Zinc acetate		20 %(w/v) PEG 3350
G3	0.2 M Sodium acetate		20 %(w/v) PEG 3350
G4	0.2 M Calcium acetate		20 %(w/v) PEG 3350
G5	0.2 M Potassium acetate		20 %(w/v) PEG 3350
G6	0.2 M Ammonium acetate		20 %(w/v) PEG 3350
G7	0.2 M Lithium sulfate		20 %(w/v) PEG 3350
G8	0.2 M Magnesium sulfate		20 %(w/v) PEG 3350

G9	0.2 M Sodium sulfate		20 %(w/v) PEG 3350
G10	0.2 M Potassium sulfate		20 %(w/v) PEG 3350
G11	0.2 M Ammonium sulfate		20 %(w/v) PEG 3350
G12	0.2 M di-Sodium tartrate		20 %(w/v) PEG 3350
H1	0.2 M K/Na tartrate		20 %(w/v) PEG 3350
H2	0.2 M di-Ammonium tartrate		20 %(w/v) PEG 3350
H3	0.2 M Sodium phosphate		20 %(w/v) PEG 3350
H4	0.2 M di-Sodium phosphate		20 %(w/v) PEG 3350
H5	0.2 M Potassium phosphate		20 %(w/v) PEG 3350
H6	0.2 M di-Potassium phosphate		20 %(w/v) PEG 3350
H7	0.2 M Ammonium phosphate		20 %(w/v) PEG 3350
H8	0.2 M di-Ammonium phosphate		20 %(w/v) PEG 3350
H9	0.2 M tri-Lithium citrate		20 %(w/v) PEG 3350
H10	0.2 M tri-Sodium citrate		20 %(w/v) PEG 3350
H11	0.2 M tri-Potassium citrate		20 %(w/v) PEG 3350
H12	0.18 M tri-Ammonium citrate		20 %(w/v) PEG 3350

MPD Suite

Well	Salt	Buffer	Precipitan
A1	0.2 M Cadmium chloride		40 %(v/v) MPD
A2	0.2 M Potassium fluoride		40 %(v/v) MPD
A3	0.2 M Ammonium fluoride		40 %(v/v) MPD
A4	0.2 M Lithium chloride		40 %(v/v) MPD
A5	0.2 M Magnesium chloride		40 %(v/v) MPD
A6	0.2 M Sodium chloride		40 %(v/v) MPD
A7	0.2 M Calcium chloride		40 %(v/v) MPD
A8	0.2 M Potassium chloride		40 %(v/v) MPD
A9	0.2 M Ammonium chloride		40 %(v/v) MPD
A10	0.2 M Sodium iodide		40 %(v/v) MPD
A11	0.2 M Potassium iodide		40 %(v/v) MPD
A12	0.2 M Ammonium iodide		40 %(v/v) MPD
B1	0.2 M Sodium thiocyanate		40 %(v/v) MPD
B2	0.2 M Potassium thiocyanate		40 %(v/v) MPD
B3	0.2 M Lithium nitrate		40 %(v/v) MPD
B4	0.2 M Magnesium nitrate		40 %(v/v) MPD
B5	0.2 M Sodium nitrate		40 %(v/v) MPD
B6	0.2 M Potassium nitrate		40 %(v/v) MPD
B7	0.2 M Ammonium nitrate		40 %(v/v) MPD
B8	0.2 M Zinc sulfate		40 %(v/v) MPD
B9	0.2 M Sodium formate		40 %(v/v) MPD
B10	0.2 M Potassium formate		40 %(v/v) MPD
B11	0.2 M Ammonium formate		40 %(v/v) MPD
B12	0.2 M Lithium acetate		40 %(v/v) MPD
C1	0.2 M Magnesium acetate		40 %(v/v) MPD
C2	0.2 M Sodium malonate		40 %(v/v) MPD
C3	0.2 M Sodium acetate		40 %(v/v) MPD
C4	0.2 M Calcium acetate		40 %(v/v) MPD
C5	0.2 M Potassium acetate		40 %(v/v) MPD
C6	0.2 M Ammonium acetate		40 %(v/v) MPD
C7	0.2 M Lithium sulfate		40 %(v/v) MPD
C8	0.2 M Magnesium sulfate		40 %(v/v) MPD
C9	0.2 M Cesium chloride		40 %(v/v) MPD

C10	0.2 M Nickel chloride		40 %(v/v) MPD
C11	0.2 M Ammonium sulfate		40 %(v/v) MPD
C12	0.2 M di-Sodium tartrate		40 %(v/v) MPD
D1	0.2 M Potassium/Sodium tartrate		40 %(v/v) MPD
D2	0.2 M di-Ammonium tartrate		40 %(v/v) MPD
D3	0.2 M Sodium phosphate		40 %(v/v) MPD
D4	0.2 M Potassium bromide		40 %(v/v) MPD
D5	0.2 M Sodium bromide		40 %(v/v) MPD
D6	0.2 M di-Potassium phosphate		40 %(v/v) MPD
D7	0.2 M Ammonium phosphate		40 %(v/v) MPD
D8	0.2 M di-Ammonium phosphate		40 %(v/v) MPD
D9	0.2 M tri-Lithium citrate		40 %(v/v) MPD
D10	0.2 M Sodium citrate		40 %(v/v) MPD
D11	0.2 M tri-Potassium citrate		40 %(v/v) MPD
D12	0.18 M tri-Ammonium citrate		40 %(v/v) MPD
E1		0.1 M Citric acid pH 4.0	10 %(v/v) MPD
E2		0.1 M Sodium acetate pH 5.0	10 %(v/v) MPD
E3		0.1 M MES pH 6.0	10 %(v/v) MPD
E4		0.1 M HEPES pH 7.0	10 %(v/v) MPD
E5		0.1 M TRIS pH 8.0	10 %(v/v) MPD
E6		0.1 M BICINE pH 9.0	10 %(v/v) MPD
E7		0.1 M Citric acid pH 4.0	20 %(v/v) MPD
E8		0.1 M Sodium acetate pH 5.0	20 %(v/v) MPD
E9		0.1 M MES pH 6.0	20 %(v/v) MPD
E10		0.1 M HEPES pH 7.0	20 %(v/v) MPD
E11		0.1 M TRIS pH 8.0	20 %(v/v) MPD
E12		0.1 M BICINE pH 9.0	20 %(v/v) MPD
F1		0.1 M Citric acid pH 4.0	40 %(v/v) MPD
F2		0.1 M Sodium acetate pH 5.0	40 %(v/v) MPD
F3		0.1 M MES pH 6.0	40 %(v/v) MPD
F4		0.1 M HEPES pH 7.0	40 %(v/v) MPD
F5		0.1 M TRIS pH 8.0	40 %(v/v) MPD
F6		0.1 M BICINE pH 9.0	40 %(v/v) MPD
F7		0.1 M Sodium acetate pH 4.0	65 %(v/v) MPD
F8		0.1 M Sodium acetate pH 5.0	65 %(v/v) MPD
F9		0.1 M MES pH 6.0	65 %(v/v) MPD
F10		0.1 M HEPES pH 7.0	65 %(v/v) MPD
F11		0.1 M TRIS pH 8.0	65 %(v/v) MPD
F12		0.1 M BICINE pH 9.0	65 %(v/v) MPD
G1	0.1 M Sodium citrate	0.1 M HEPES sodium salt pH 7.5	10 %(w/v) MPD
G2	0.05 M Magnesium chloride	0.1 M TRIS.HCl pH 8.5	12 %(w/v) MPD
G3	0.02 M Calcium chloride	0.1 M Sodium acetate pH 4.6	15 %(w/v) MPD
G4		0.1 M Imidazole.HCl pH 8.0	15 %(w/v) MPD, 5 %(w/v) PEG 4000
G5	0.2 M Ammonium acetate	0.1 M Sodium citrate pH 5.6	15 %(w/v) MPD
G6	0.2 M Magnesium acetate	0.1 M MES sodium salt pH 6.5	15 %(w/v) MPD
G7	0.2 M Sodium citrate	0.1 M HEPES sodium salt pH 7.5	15 %(w/v) MPD
G8	0.1 M Sodium citrate	0.1 M HEPES sodium salt pH 7.5	20 %(w/v) MPD
G9		0.1 M Imidazole.HCl pH 8.0	20 %(w/v) MPD
G10	0.2 M Sodium chloride		20 %(w/v) MPD, 4 %(w/v) Glycerol
G11	0.02 M Calcium chloride	0.1 M Sodium acetate pH 4.6	30 %(w/v) MPD
G12	0.2 M Ammonium acetate	0.1 M Sodium citrate pH 5.6	30 %(w/v) MPD

H1	0.2 M Magnesium acetate	0.1 M MES sodium salt pH 6.5	30 %(w/v) MPD
H2	0.5 M Ammonium sulfate	0.1 M HEPES sodium salt pH 7.5	30 %(w/v) MPD
H3	0.2 M Sodium citrate	0.1 M HEPES sodium salt pH 7.5	30 %(w/v) MPD
H4		0.1 M HEPES sodium salt pH 7.5	30 %(w/v) MPD, 5 %(w/v) PEG 4000
H5		0.1 M Imidazole.HCl pH 8.0	30 %(w/v) MPD, 10 %(w/v) PEG 4000
H6			30 %(w/v) MPD, 20 %(w/v) Ethanol
H7			35 %(w/v) MPD
H8		0.1 M Imidazole.HCl pH 8.0	35 %(w/v) MPD
H9		0.1 M TRIS.HCl pH 8.5	40 %(w/v) MPD
H10		0.1 M HEPES sodium salt pH 7.5	47 %(w/v) MPD
H11			47 %(w/v) MPD, 2 %(w/v) tert-Butanol
H12			50 %(w/v) MPD

pH Clear Suite

Well	Buffer	Precipitant	Final pH
A1	0.1 M Citric acid	1.0 M Sodium chloride	4.0
A2	0.1 M Citric acid	1.0 M Sodium chloride	5.0
A3	0.1 M MES	1.0 M Sodium chloride	6.0
A4	0.1 M HEPES	1.0 M Sodium chloride	7.0
A5	0.1 M TRIS	1.0 M Sodium chloride	8.0
A6	0.1 M BICINE	1.0 M Sodium chloride	9.0
A7	0.1 M Citric acid	2.0 M Sodium chloride	4.0
A8	0.1 M Citric acid	2.0 M Sodium chloride	5.0
A9	0.1 M MES	2.0 M Sodium chloride	6.0
A10	0.1 M HEPES	2.0 M Sodium chloride	7.0
A11	0.1 M TRIS	2.0 M Sodium chloride	8.0
A12	0.1 M BICINE	2.0 M Sodium chloride	9.0
B1	0.1 M Citric acid	3.0 M Sodium chloride	4.0
B2	0.1 M Citric acid	3.0 M Sodium chloride	5.0
B3	0.1 M MES	3.0 M Sodium chloride	6.0
B4	0.1 M HEPES	3.0 M Sodium chloride	7.0
B5	0.1 M TRIS	3.0 M Sodium chloride	8.0
B6	0.1 M BICINE	3.0 M Sodium chloride	9.0
B7	0.1 M Citric acid	4.0 M Sodium chloride	4.0
B8	0.1 M Citric acid	4.0 M Sodium chloride	5.0
B9	0.1 M MES	4.0 M Sodium chloride	6.0
B10	0.1 M HEPES	4.0 M Sodium chloride	7.0
B11	0.1 M TRIS	4.0 M Sodium chloride	8.0
B12	0.1 M BICINE	4.0 M Sodium chloride	9.0
C1	0.1 M Citric acid	5 %(w/v) PEG 6000	4.0
C2	0.1 M Citric acid	5 %(w/v) PEG 6000	5.0
C3	0.1 M MES	5 %(w/v) PEG 6000	6.0
C4	0.1 M HEPES	5 %(w/v) PEG 6000	7.0
C5	0.1 M TRIS	5 %(w/v) PEG 6000	8.0
C6	0.1 M BICINE	5 %(w/v) PEG 6000	9.0
C7	0.1 M Citric acid	10 %(w/v) PEG 6000	4.0
C8	0.1 M Citric acid	10 %(w/v) PEG 6000	5.0
C9	0.1 M MES	10 %(w/v) PEG 6000	6.0
C10	0.1 M HEPES	10 %(w/v) PEG 6000	7.0
C11	0.1 M TRIS	10 %(w/v) PEG 6000	8.0
C12	0.1 M BICINE	10 %(w/v) PEG 6000	9.0

D1	0.1 M Citric acid	20 %(w/v) PEG 6000	4.0
D2	0.1 M Citric acid	20 %(w/v) PEG 6000	5.0
D3	0.1 M MES	20 %(w/v) PEG 6000	6.0
D4	0.1 M HEPES	20 %(w/v) PEG 6000	7.0
D5	0.1 M TRIS	20 %(w/v) PEG 6000	8.0
D6	0.1 M BICINE	20 %(w/v) PEG 6000	9.0
D7	0.1 M Citric acid	30 %(w/v) PEG 6000	4.0
D8	0.1 M Citric acid	30 %(w/v) PEG 6000	5.0
D9	0.1 M MES	30 %(w/v) PEG 6000	6.0
D10	0.1 M HEPES	30 %(w/v) PEG 6000	7.0
D11	0.1 M TRIS	30 %(w/v) PEG 6000	8.0
D12	0.1 M BICINE	30 %(w/v) PEG 6000	9.0
E1	0.1 M Citric acid	0.8 M Ammonium sulfate	4.0
E2	0.1 M Citric acid	0.8 M Ammonium sulfate	5.0
E3	0.1 M MES	0.8 M Ammonium sulfate	6.0
E4	0.1 M HEPES	0.8 M Ammonium sulfate	7.0
E5	0.1 M TRIS	0.8 M Ammonium sulfate	8.0
E6	0.1 M BICINE	0.8 M Ammonium sulfate	9.0
E7	0.1 M Citric acid	1.6 M Ammonium sulfate	4.0
E8	0.1 M Citric acid	1.6 M Ammonium sulfate	5.0
E9	0.1 M MES	1.6 M Ammonium sulfate	6.0
E10	0.1 M HEPES	1.6 M Ammonium sulfate	7.0
E11	0.1 M TRIS	1.6 M Ammonium sulfate	8.0
E12	0.1 M BICINE	1.6 M Ammonium sulfate	9.0
F1	0.1 M Citric acid	2.4 M Ammonium sulfate	4.0
F2	0.1 M Citric acid	2.4 M Ammonium sulfate	5.0
F3	0.1 M MES	2.4 M Ammonium sulfate	6.0
F4	0.1 M HEPES	2.4 M Ammonium sulfate	7.0
F5	0.1 M TRIS	2.4 M Ammonium sulfate	8.0
F6	0.1 M BICINE	2.4 M Ammonium sulfate	9.0
F7	0.1 M Citric acid	3.2 M Ammonium sulfate	4.0
F8	0.1 M Citric acid	3.2 M Ammonium sulfate	5.0
F9	0.1 M MES	3.2 M Ammonium sulfate	6.0
F10	0.1 M HEPES	3.2 M Ammonium sulfate	7.0
F11	0.1 M TRIS	3.2 M Ammonium sulfate	8.0
F12	0.1 M BICINE	3.2 M Ammonium sulfate	9.0
G1	0.1 M Citric acid	10 %(v/v) MPD	4.0
G2	0.1 M Sodium acetate	10 %(v/v) MPD	5.0
G3	0.1 M MES	10 %(v/v) MPD	6.0
G4	0.1 M HEPES	10 %(v/v) MPD	7.0
G5	0.1 M TRIS	10 %(v/v) MPD	8.0
G6	0.1 M BICINE	10 %(v/v) MPD	9.0
G7	0.1 M Citric acid	20 %(v/v) MPD	4.0
G8	0.1 M Sodium acetate	20 %(v/v) MPD	5.0
G9	0.1 M MES	20 %(v/v) MPD	6.0
G10	0.1 M HEPES	20 %(v/v) MPD	7.0
G11	0.1 M TRIS	20 %(v/v) MPD	8.0
G12	0.1 M BICINE	20 %(v/v) MPD	9.0
H1	0.1 M Citric acid	40 %(v/v) MPD	4.0
H2	0.1 M Sodium acetate	40 %(v/v) MPD	5.0
H3	0.1 M MES	40 %(v/v) MPD	6.0
H4	0.1 M HEPES	40 %(v/v) MPD	7.0

H5	0.1 M TRIS	40 %(v/v) MPD	8.0
H6	0.1 M BICINE	40 %(v/v) MPD	9.0
H7	0.1 M Citric acid	65 %(v/v) MPD	4.0
H8	0.1 M Sodium acetate	65 %(v/v) MPD	5.0
H9	0.1 M MES	65 %(v/v) MPD	6.0
H10	0.1 M HEPES	65 %(v/v) MPD	7.0
H11	0.1 M TRIS	65 %(v/v) MPD	8.0
H12	0.1 M BICINE	65 %(v/v) MPD	9.0

pH Clear II Suite

Well	Salt	Buffer	Precipitant	Final pH
A1	1.0 M Lithium chloride	0.1 M Citric acid		4.0
A2	1.0 M Lithium chloride	0.1 M Citric acid		5.0
A3	1.0 M Lithium chloride	0.1 M MES		6.0
A4	1.0 M Lithium chloride	0.1 M HEPES		7.0
A5	1.0 M Lithium chloride	0.1 M TRIS		8.0
A6	1.0 M Lithium chloride	0.1 M BICINE		9.0
A7	1.0 M Lithium chloride	0.1 M Citric acid	10 %(w/v) PEG 6000	4.0
A8	1.0 M Lithium chloride	0.1 M Citric acid	10 %(w/v) PEG 6000	5.0
A9	1.0 M Lithium chloride	0.1 M MES	10 %(w/v) PEG 6000	6.0
A10	1.0 M Lithium chloride	0.1 M HEPES	10 %(w/v) PEG 6000	7.0
A11	1.0 M Lithium chloride	0.1 M TRIS	10 %(w/v) PEG 6000	8.0
A12	1.0 M Lithium chloride	0.1 M BICINE	10 %(w/v) PEG 6000	9.0
B1	1.0 M Lithium chloride	0.1 M Citric acid	20 %(w/v) PEG 6000	4.0
B2	1.0 M Lithium chloride	0.1 M Citric acid	20 %(w/v) PEG 6000	5.0
B3	1.0 M Lithium chloride	0.1 M MES	20 %(w/v) PEG 6000	6.0
B4	1.0 M Lithium chloride	0.1 M HEPES	20 %(w/v) PEG 6000	7.0
B5	1.0 M Lithium chloride	0.1 M TRIS	20 %(w/v) PEG 6000	8.0
B6	1.0 M Lithium chloride	0.1 M BICINE	20 %(w/v) PEG 6000	9.0
B7	1.0 M Lithium chloride	0.1 M Citric acid	30 %(w/v) PEG 6000	4.0
B8	1.0 M Lithium chloride	0.1 M Citric acid	30 %(w/v) PEG 6000	5.0
B9	1.0 M Lithium chloride	0.1 M MES	30 %(w/v) PEG 6000	6.0
B10	1.0 M Lithium chloride	0.1 M HEPES	30 %(w/v) PEG 6000	7.0
B11	1.0 M Lithium chloride	0.1 M TRIS	30 %(w/v) PEG 6000	8.0
B12	1.0 M Lithium chloride	0.1 M BICINE	30 %(w/v) PEG 6000	9.0
C1		0.1 M Citric acid	5 %(v/v) Isopropanol	4.0
C2		0.1 M Citric acid	5 %(v/v) Isopropanol	5.0
C3		0.1 M MES	5 %(v/v) Isopropanol	6.0
C4		0.1 M HEPES	5 %(v/v) Isopropanol	7.0
C5		0.1 M TRIS	5 %(v/v) Isopropanol	8.0
C6		0.1 M BICINE	5 %(v/v) Isopropanol	9.0
C7		0.1 M Citric acid	10 %(v/v) Isopropanol	4.0
C8		0.1 M Citric acid	10 %(v/v) Isopropanol	5.0
C9		0.1 M MES	10 %(v/v) Isopropanol	6.0
C10		0.1 M HEPES	10 %(v/v) Isopropanol	7.0
C11		0.1 M TRIS	10 %(v/v) Isopropanol	8.0
C12		0.1 M BICINE	10 %(v/v) Isopropanol	9.0
D1		0.1 M Citric acid	20 %(v/v) Isopropanol	4.0
D2		0.1 M Citric acid	20 %(v/v) Isopropanol	5.0
D3		0.1 M MES	20 %(v/v) Isopropanol	6.0
D4		0.1 M HEPES	20 %(v/v) Isopropanol	7.0
D5		0.1 M TRIS	20 %(v/v) Isopropanol	8.0

D6		0.1 M BICINE	20 %(v/v) Isopropanol	9.0
D7		0.1 M Citric acid	30 %(v/v) Isopropanol	4.0
D8		0.1 M Citric acid	30 %(v/v) Isopropanol	5.0
D9		0.1 M MES	30 %(v/v) Isopropanol	6.0
D10		0.1 M HEPES	30 %(v/v) Isopropanol	7.0
D11		0.1 M TRIS	30 %(v/v) Isopropanol	8.0
D12		0.1 M BICINE	30 %(v/v) Isopropanol	9.0
E1			0.8 M Na/K phosphate	5.0
E2			0.8 M Na/K phosphate	5.6
E3			0.8 M Na/K phosphate	6.3
E4			0.8 M Na/K phosphate	6.9
E5			0.8 M Na/K phosphate	7.5
E6			0.8 M Na/K phosphate	8.2
E7			1.0 M Na/K phosphate	5.0
E8			1.0 M Na/K phosphate	5.6
E9			1.0 M Na/K phosphate	6.3
E10			1.0 M Na/K phosphate	6.9
E11			1.0 M Na/K phosphate	7.5
E12			1.0 M Na/K phosphate	8.2
F1			1.4 M Na/K phosphate	5.0
F2			1.4 M Na/K phosphate	5.6
F3			1.4 M Na/K phosphate	6.3
F4			1.4 M Na/K phosphate	6.9
F5			1.4 M Na/K phosphate	7.5
F6			1.4 M Na/K phosphate	8.2
F7			1.8 M Na/K phosphate	5.0
F8			1.8 M Na/K phosphate	5.6
F9			1.8 M Na/K phosphate	6.3
F10			1.8 M Na/K phosphate	6.9
F11			1.8 M Na/K phosphate	7.5
F12			1.8 M Na/K phosphate	8.2
G1			1.0 M Sodium malonate	4.0
G2			1.5 M Sodium malonate	4.0
G3			1.9 M Sodium malonate	4.0
G4			2.4 M Sodium malonate	4.0
G5			2.9 M Sodium malonate	4.0
G6			3.4 M Sodium malonate	4.0
G7			1.0 M Sodium malonate	5.0
G8			1.5 M Sodium malonate	5.0
G9			1.9 M Sodium malonate	5.0
G10			2.4 M Sodium malonate	5.0
G11			2.9 M Sodium malonate	5.0
G12			3.4 M Sodium malonate	5.0
H1			1.0 M Sodium malonate	6.0
H2			1.5 M Sodium malonate	6.0
H3			1.9 M Sodium malonate	6.0
H4			2.4 M Sodium malonate	6.0
H5			2.9 M Sodium malonate	6.0
H6			3.4 M Sodium malonate	6.0
H7			1.0 M Sodium malonate	7.0
H8			1.5 M Sodium malonate	7.0
H9			1.9 M Sodium malonate	7.0

H10			2.4 M Sodium malonate	7.0
H11			2.9 M Sodium malonate	7.0
H12			3.4 M Sodium malonate	7.0

PACT Suite

Well	Salt	Buffer	Precipitant
A1		0.1M SPG buffer pH 4	25% w/v PEG 1500
A2		0.1M SPG buffer pH 5	25% w/v PEG 1500
A3		0.1M SPG buffer pH 6	25% w/v PEG 1500
A4		0.1M SPG buffer pH 7	25% w/v PEG 1500
A5		0.1M SPG buffer pH 8	25% w/v PEG 1500
A6		0.1M SPG buffer pH 9	25% w/v PEG 1500
A7	0.2M Sodium chloride	0.1M Sodium acetate pH 5	20% w/v PEG 6000
A8	0.2M Ammonium chloride	0.1M Sodium acetate pH 5	20% w/v PEG 6000
A9	0.2M Lithium chloride	0.1M Sodium acetate pH 5	20% w/v PEG 6000
A10	0.2M Magnesium chloride	0.1M Sodium acetate pH 5	20% w/v PEG 6000
A11	0.2M Calcium chloride	0.1M Sodium acetate pH 5	20% w/v PEG 6000
A12	0.01M Zinc chloride	0.1M Sodium acetate pH 5	20% w/v PEG 6000
B1		0.1M MIB buffer pH 4	25% w/v PEG 1500
B2		0.1M MIB buffer pH 5	25% w/v PEG 1500
B3		0.1M MIB buffer pH 6	25% w/v PEG 1500
B4		0.1M MIB buffer pH 7	25% w/v PEG 1500
B5		0.1M MIB buffer pH 8	25% w/v PEG 1500
B6		0.1M MIB buffer pH 9	25% w/v PEG 1500
B7	0.2M Sodium chloride	0.1M MES pH 6	20% w/v PEG 6000
B8	0.2M Ammonium chloride	0.1M MES pH 6	20% w/v PEG 6000
B9	0.2M Lithium chloride	0.1M MES pH 6	20% w/v PEG 6000
B10	0.2M Magnesium chloride	0.1M MES pH 6	20% w/v PEG 6000
B11	0.2M Calcium chloride	0.1M MES pH 6	20% w/v PEG 6000
B12	0.01M Zinc chloride	0.1M MES pH 6	20% w/v PEG 6000
C1		0.1M PCB buffer pH 4	25% w/v PEG 1500
C2		0.1M PCB buffer pH 5	25% w/v PEG 1500
C3		0.1M PCB buffer pH 6	25% w/v PEG 1500
C4		0.1M PCB buffer pH 7	25% w/v PEG 1500
C5		0.1M PCB buffer pH 8	25% w/v PEG 1500
C6		0.1M PCB buffer pH 9	25% w/v PEG 1500
C7	0.2M Sodium chloride	0.1M Hepes pH 7	20% w/v PEG 6000
C8	0.2M Ammonium chloride	0.1M Hepes pH 7	20% w/v PEG 6000
C9	0.2M Lithium chloride	0.1M Hepes pH 7	20% w/v PEG 6000
C10	0.2M Magnesium chloride	0.1M Hepes pH 7	20% w/v PEG 6000
C11	0.2M Calcium chloride	0.1M Hepes pH 7	20% w/v PEG 6000
C12	0.01M Zinc chloride	0.1M Hepes pH 7	20% w/v PEG 6000
D1		0.1M MMT buffer pH 4	25% w/v PEG 1500
D2		0.1M MMT buffer pH 5	25% w/v PEG 1500
D3		0.1M MMT buffer pH 6	25% w/v PEG 1500
D4		0.1M MMT buffer pH 7	25% w/v PEG 1500
D5		0.1M MMT buffer pH 8	25% w/v PEG 1500
D6		0.1M MMT buffer pH 9	25% w/v PEG 1500
D7	0.2M Sodium chloride	0.1M Tris pH 8	20% w/v PEG 6000
D8	0.2M Ammonium chloride	0.1M Tris pH 8	20% w/v PEG 6000
D9	0.2M Lithium chloride	0.1M Tris pH 8	20% w/v PEG 6000
D10	0.2M Magnesium chloride	0.1M Tris pH 8	20% w/v PEG 6000

D11	0.2M Calcium chloride	0.1M Tris pH 8	20% w/v PEG 6000
D12		0.1M Tris pH 8	20% w/v PEG 6000
E1	0.2M Sodium fluoride		20% w/v PEG 3350
E2	0.2M Sodium bromide		20% w/v PEG 3350
E3	0.2M Sodium iodide		20% w/v PEG 3350
E4	0.2M Potassium thiocyanate		20% w/v PEG 3350
E5	0.2M Sodium nitrate		20% w/v PEG 3350
E6	0.2M Sodium formate		20% w/v PEG 3350
E7	0.2M Sodium acetate		20% w/v PEG 3350
E8	0.2M Sodium sulphate		20% w/v PEG 3350
E9	0.2M Potassium/sodium tartrate		20% w/v PEG 3350
E10	0.2M Sodium/potassium phosphate		20% w/v PEG 3350
E11	0.2M Sodium citrate		20% w/v PEG 3350
E12	0.2M Sodium malonate		20% w/v PEG 3350
F1	0.2M Sodium fluoride	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F2	0.2M Sodium bromide	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F3	0.2M Sodium iodide	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F4	0.2M Potassium thiocyanate	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F5	0.2M Sodium nitrate	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F6	0.2M Sodium formate	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F7	0.2M Sodium acetate	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F8	0.2M Sodium sulphate	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F9	0.2M Potassium/sodium tartrate	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F10	0.2M Sodium/potassium phosphate	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F11	0.2M Sodium citrate	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
F12	0.2M Sodium malonate	0.1M Bis Tris propane pH 6.5	20% w/v PEG 3350
G1	0.2M Sodium fluoride	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G2	0.2M Sodium bromide	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G3	0.2M Sodium iodide	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G4	0.2M Potassium thiocyanate	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G5	0.2M Sodium nitrate	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G6	0.2M Sodium formate	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G7	0.2M Sodium acetate	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G8	0.2M Sodium sulphate	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G9	0.2M Potassium/sodium tartarte	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G10	0.2M Sodium/potassium phosphate	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G11	0.2M Sodium citrate	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
G12	0.2M Sodium malonate	0.1M Bis Tris propane pH 7.5	20% w/v PEG 3350
H1	0.2M Sodium fluoride	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H2	0.2M Sodium bromide	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H3	0.2M Sodium iodide	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H4	0.2M Potassium thiocyanate	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H5	0.2M Sodium nitrate	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H6	0.2M Sodium formate	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H7	0.2M Sodium acetate	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H8	0.2M Sodium sulphate	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H9	0.2M Potassium/sodium tartrate	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H10	0.2M Sodium/potassium phosphate	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H11	0.2M Sodium citrate	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350
H12	0.2M Sodium malonate	0.1M Bis Tris propane pH 8.5	20% w/v PEG 3350

HR2-086

Well	Buffer	Additive/Salt	Polymer
A1	0.1 M Citric acid pH 3.5		34% v/v Polyethylene glycol 200
A2	0.1 M Sodium citrate tribasic dihydrate pH 5.5		38% v/v Polyethylene glycol 200
A3	0.1 M HEPES pH 7.5		42% v/v Polyethylene glycol 200
A4	0.1 M Sodium acetate trihydrate pH 4.5		30% v/v Polyethylene glycol 300
A5	0.1 M BIS-TRIS pH 6.5		25% v/v Polyethylene glycol 300
A6	0.1 M BICINE pH 8.5		20% v/v Polyethylene glycol 300
A7	0.1 M Sodium acetate trihydrate pH 4.0		15% v/v Polyethylene glycol 400
A8	0.1 M MES monohydrate pH 6.0		22% v/v Polyethylene glycol 400
A9	0.1 M Tris pH 8.0		30% v/v Polyethylene glycol 400
A10	0.1 M Sodium citrate tribasic dihydrate pH 5.0		30% v/v Polyethylene glycol monomethyl ether 550
A11	0.1 M Imidazole pH 7.0		25% v/v Polyethylene glycol monomethyl ether 550
A12	0.1 M BIS-TRIS propane pH 9.0		20% v/v Polyethylene glycol monomethyl ether 550
B1	0.1 M Sodium acetate trihydrate pH 4.0		10% v/v Jeffamine® M-600® pH 7.0
B2	0.1 M MES monohydrate pH 6.0		20% v/v Jeffamine® M-600® pH 7.0
B3	0.1 M Tris pH 8.0		30% v/v Jeffamine® M-600® pH 7.0
B4	0.1 M Citric acid pH 3.5		14% w/v Polyethylene glycol 1,000
B5	0.1 M Sodium citrate tribasic dihydrate pH 5.5		22% w/v Polyethylene glycol 1,000
B6	0.1 M HEPES pH 7.5		30% w/v Polyethylene glycol 1,000
B7	0.1 M Sodium acetate trihydrate pH 4.5		30% w/v Polyethylene glycol 1,500
B8	0.1 M BIS-TRIS pH 6.5		20% w/v Polyethylene glycol 1,500
B9	0.1 M BICINE pH 8.5		15% w/v Polyethylene glycol 1,500
B10	0.1 M Sodium acetate trihydrate pH 4.0		10% w/v Polyethylene glycol monomethyl ether 2,000
B11	0.1 M MES monohydrate pH 6.0		20% w/v Polyethylene glycol monomethyl ether 2,000
B12	0.1 M Tris pH 8.0		30% w/v Polyethylene glycol monomethyl ether 2,000
C1	0.1 M Sodium citrate tribasic dihydrate pH 5.0		30% v/v Jeffamine® ED-2001 pH 7.0
C2	0.1 M Imidazole pH 7.0		20% v/v Jeffamine® ED-2001 pH 7.0
C3	0.1 M BIS-TRIS propane pH 9.0		10% v/v Jeffamine® ED-2001 pH 7.0
C4	0.1 M Citric acid pH 3.5		25% w/v Polyethylene glycol 3,350
C5	0.1 M Sodium citrate tribasic dihydrate pH 5.5		18% w/v Polyethylene glycol 3,350
C6	0.1 M HEPES pH 7.5		12% w/v Polyethylene glycol 3,350
C7	0.1 M Sodium acetate trihydrate pH 4.0		10% w/v Polyethylene glycol 4,000
C8	0.1 M MES monohydrate pH 6.0		14% w/v Polyethylene glycol 4,000
C9	0.1 M Tris pH 8.0		28% w/v Polyethylene glycol 4,000
C10	0.1 M Sodium acetate trihydrate pH 4.5		30% w/v Polyethylene glycol monomethyl ether 5,000
C11	0.1 M BIS-TRIS pH 6.5		20% w/v Polyethylene glycol monomethyl ether 5,000
C12	0.1 M BICINE pH 8.5		8% w/v Polyethylene glycol monomethyl ether 5,000
D1	0.1 M Sodium citrate tribasic dihydrate pH 5.0		10% w/v Polyethylene glycol 6,000
D2	0.1 M Imidazole pH 7.0		20% w/v Polyethylene glycol 6,000
D3	0.1 M BIS-TRIS propane pH 9.0		30% w/v Polyethylene glycol 6,000
D4	0.1 M Citric acid pH 3.5		28% w/v Polyethylene glycol 8,000
D5	0.1 M Sodium citrate tribasic dihydrate pH 5.5		16% w/v Polyethylene glycol 8,000
D6	0.1 M HEPES pH 7.5		4% w/v Polyethylene glycol 8,000
D7	0.1 M Sodium acetate trihydrate pH 4.5		10% w/v Polyethylene glycol 10,000

D8	0.1 M BIS-TRIS pH 6.5		16% w/v Polyethylene glycol 10,000
D9	0.1 M BICINE pH 8.5		20% w/v Polyethylene glycol 10,000
D10	0.1 M Sodium citrate tribasic dihydrate pH 5.0		18% w/v Polyethylene glycol 20,000
D11	0.1 M Imidazole pH 7.0		12% w/v Polyethylene glycol 20,000
D12	0.1 M BIS-TRIS propane pH 9.0		8% w/v Polyethylene glycol 20,000
E1	0.1 M Sodium acetate trihydrate pH 4.0	0.8 M Lithium sulfate monohydrate	4% v/v Polyethylene glycol 200
E2	0.1 M Sodium citrate tribasic dihydrate pH 5.0	0.2 M Lithium sulfate monohydrate	26% v/v Polyethylene glycol 200
E3	0.1 M MES monohydrate pH 6.0	0.05 M Calcium chloride dihydrate	45% v/v Polyethylene glycol 200
E4	0.1 M BIS-TRIS pH 6.5	28% v/v 2-Propanol	3% v/v Polyethylene glycol 200
E5	0.1 M HEPES pH 7.5	20% v/v Tacsimate pH 7.0	2% v/v Polyethylene glycol 200
E6	0.1 M Sodium citrate tribasic dihydrate pH 5.0	10% v/v 2-Propanol	26% v/v Polyethylene glycol 400
E7	0.1 M Sodium citrate tribasic dihydrate pH 5.5	0.2 M Ammonium acetate	24% v/v Polyethylene glycol 400
E8	0.1 M BIS-TRIS pH 6.5	0.2 M Ammonium sulfate	18% v/v Polyethylene glycol 400
E9	0.1 M HEPES pH 7.5	0.19 mM CYMAL®-7	40% v/v Polyethylene glycol 400
E10	0.1 M Sodium acetate trihydrate pH 4.5	6% v/v 2-Propanol	26% v/v Polyethylene glycol monomethyl ether 550
E11	0.1 M BIS-TRIS pH 6.5	1.8 M Ammonium sulfate	2% v/v Polyethylene glycol monomethyl ether 550
E12	0.1 M Imidazole pH 7.0	0.15 M DL-Malic acid pH 7.0	22% v/v Polyethylene glycol monomethyl ether 550
F1	0.1 M BICINE pH 8.5	0.1 M Succinic acid pH 7.0	30% v/v Polyethylene glycol monomethyl ether 550
F2	0.1 M Sodium citrate tribasic dihydrate pH 5.5	0.1 M Lithium sulfate monohydrate	20% w/v Polyethylene glycol 1,000
F3	0.1 M Tris pH 8.0	0.1 M Sodium malonate pH 8.0	30% w/v Polyethylene glycol 1,000
F4	0.1 M Citric acid pH 3.5	4% v/v (+/-)-2-Methyl-2,4-pentanediol	20% w/v Polyethylene glycol 1,500
F5	0.1 M HEPES pH 7.5	0.2 M L-Proline	24% w/v Polyethylene glycol 1,500
F6	0.1 M BICINE pH 8.5	10% v/v 2-Propanol	30% w/v Polyethylene glycol 1,500
F7	0.1 M BIS-TRIS propane pH 9.0	0.1 M Sodium chloride	25% w/v Polyethylene glycol 1,500
F8	0.1 M Sodium acetate trihydrate pH 4.5	0.02 M Nickel(II) chloride hexahydrate, 0.02 M Magnesium chloride hexahydrate, 0.02 M Cadmium chloride hydrate	24% w/v Polyethylene glycol monomethyl ether 2,000
F9	0.1 M MES monohydrate pH 6.0	20% v/v 2-Propanol	20% w/v Polyethylene glycol monomethyl ether 2,000
F10	0.1 M Imidazole pH 7.0	0.2 M Ammonium citrate tribasic pH 7.0	20% w/v Polyethylene glycol monomethyl ether 2,000
F11	0.1 M BIS-TRIS propane pH 9.0	4.0 M Potassium formate	2% w/v Polyethylene glycol monomethyl ether 2,000
F12	0.1 M Sodium acetate trihydrate pH 4.5	50% v/v Tacsimate pH 4.0	1% w/v Polyethylene glycol 3,350
G1	0.1 M Sodium citrate tribasic dihydrate pH 5.5	0.10% w/v n-Octyl-b-D-glucoside	22% w/v Polyethylene glycol 3,350
G2	0.1 M Imidazole pH 7.0	2% v/v Tacsimate pH 7.0, 5% v/v 2-Propanol	8% w/v Polyethylene glycol 3,350
G3	0.1 M Tris pH 8.0	2% v/v 1,4-Dioxane	15% w/v Polyethylene glycol 3,350
G4	0.1 M Sodium citrate tribasic dihydrate pH 5.5	18% v/v 2-Propanol	20% w/v Polyethylene glycol 4,000
G5	0.1 M MES monohydrate pH 6.0	6% v/v Tacsimate pH 6.0	25% w/v Polyethylene glycol 4,000
G6	0.1 M Sodium acetate trihydrate pH 4.0	0.2 M Magnesium formate dihydrate	18% w/v Polyethylene glycol monomethyl ether 5,000
G7	0.1 M Imidazole pH 7.0	2% v/v Polyethylene glycol 400	24% w/v Polyethylene glycol monomethyl ether 5,000
G8	0.1 M BICINE pH 8.5	0.2 M Sodium formate	20% w/v Polyethylene glycol monomethyl ether 5,000
G9	0.1 M BIS-TRIS propane pH 9.0	4% v/v 2-Propanol	20% w/v Polyethylene glycol monomethyl ether 5,000
G10	0.1 M Citric acid pH 3.5	6% v/v Ethylene glycol	10% w/v Polyethylene glycol 6,000
G11	0.1 M Citric acid pH 3.5	0.15 M Lithium sulfate monohydrate	18% w/v Polyethylene glycol 6,000

G12	0.1 M Sodium acetate trihydrate pH 4.0	10% v/v 2-Propanol	22% w/v Polyethylene glycol 6,000
H1	0.1 M Sodium acetate trihydrate pH 4.0	0.2 M Sodium chloride	22% w/v Polyethylene glycol 8,000
H2	0.1 M Tris pH 8.0	20% v/v 2-Propanol	5% w/v Polyethylene glycol 8,000
H3	0.1 M BIS-TRIS propane pH 9.0	10% v/v Polyethylene glycol 200	18% w/v Polyethylene glycol 8,000
H4	0.1 M Sodium citrate tribasic dihydrate pH 5.0	15% v/v 2-Propanol	10% w/v Polyethylene glycol 10,000
H5	0.1 M MES monohydrate pH 6.0	0.4 M Sodium malonate pH 6.0	0.5% w/v Polyethylene glycol 10,000
H6	0.1 M BIS-TRIS pH 6.5	0.2 M Potassium sodium tartrate tetrahydrate	10% w/v Polyethylene glycol 10,000
H7	0.1 M HEPES pH 7.5	5% v/v (+/-)-2-Methyl-2,4-pentanediol	10% w/v Polyethylene glycol 10,000
H8	0.1 M Tris pH 8.0	0.2 M Ammonium acetate	16% w/v Polyethylene glycol 10,000
H9	0.1 M Citric acid pH 3.5	5% v/v 2-Propanol	6% w/v Polyethylene glycol 20,000
H10	0.1 M Sodium acetate trihydrate pH 4.5	1.0 M Sodium malonate pH 5.0	2% w/v Polyethylene glycol 20,000
H11	0.1 M Sodium citrate tribasic dihydrate pH 5.0	0.2 M Magnesium chloride hexahydrate	10% w/v Polyethylene glycol 20,000
H12	0.1 M BICINE pH 8.5	3% w/v Dextran sulfate sodium salt	15% w/v Polyethylene glycol 20,000

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Well	Buffer	Salt	Precipitant
A1	0.1 M Sodium acetate trihydrate pH 4.6	0.02 M Calcium chloride dihydrate	30% v/v (+/-)-2-Methyl-2,4-pentanediol
A2	None	None	0.4 M Potassium sodium tartrate tetrahydrate
A3	None	None	0.4 M Ammonium phosphate monobasic
A4	0.1 M TRIS hydrochloride pH 8.5	None	2.0 M Ammonium sulfate
A5	0.1 M HEPES sodium pH 7.5	0.2 M Sodium citrate tribasic dihydrate	30% v/v (+/-)-2-Methyl-2,4-pentanediol
A6	0.1 M TRIS hydrochloride pH 8.5	0.2 M Magnesium chloride hexahydrate	30% w/v Polyethylene glycol 4,000
A7	0.1 M Sodium cacodylate trihydrate pH 6.5	None	1.4 M Sodium acetate trihydrate
A8	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Sodium citrate tribasic dihydrate	30% v/v 2-Propanol
A9	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.2 M Ammonium acetate	30% w/v Polyethylene glycol 4,000
A10	0.1 M Sodium acetate trihydrate pH 4.6	0.2 M Ammonium acetate	30% w/v Polyethylene glycol 4,000
A11	0.1 M Sodium citrate tribasic dihydrate pH 5.6	None	1.0 M Ammonium phosphate monobasic
A12	0.1 M HEPES sodium pH 7.5	0.2 M Magnesium chloride hexahydrate	30% v/v 2-Propanol
B1	0.1 M TRIS hydrochloride pH 8.5	0.2 M Sodium citrate tribasic dihydrate	30% v/v Polyethylene glycol 400
B2	0.1 M HEPES sodium pH 7.5	0.2 M Calcium chloride dihydrate	28% v/v Polyethylene glycol 400
B3	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Ammonium sulfate	30% w/v Polyethylene glycol 8,000
B4	0.1 M HEPES sodium pH 7.5	None	1.5 M Lithium sulfate monohydrate
B5	0.1 M TRIS hydrochloride pH 8.5	0.2 M Lithium sulfate monohydrate	30% w/v Polyethylene glycol 4,000
B6	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Magnesium acetate tetrahydrate	20% w/v Polyethylene glycol 8,000
B7	0.1 M TRIS hydrochloride pH 8.5	0.2 M Ammonium acetate	30% v/v 2-Propanol
B8	0.1 M Sodium acetate trihydrate pH 4.6	0.2 M Ammonium sulfate	25% w/v Polyethylene glycol 4,000
B9	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Magnesium acetate tetrahydrate	30% v/v (+/-)-2-Methyl-2,4-pentanediol
B10	0.1 M TRIS hydrochloride pH 8.5	0.2 M Sodium acetate trihydrate	30% w/v Polyethylene glycol 4,000
B11	0.1 M HEPES sodium pH 7.5	0.2 M Magnesium chloride hexahydrate	30% v/v Polyethylene glycol 400
B12	0.1 M Sodium acetate trihydrate pH 4.6	0.2 M Calcium chloride dihydrate	20% v/v 2-Propanol
C1	0.1 M Imidazole pH 6.5	None	1.0 M Sodium acetate trihydrate

C2	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.2 M Ammonium acetate	30% v/v (+/-)-2-Methyl-2,4-pentanediol
C3	0.1 M HEPES sodium pH 7.5	0.2 M Sodium citrate tribasic dihydrate	20% v/v 2-Propanol
C4	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Sodium acetate trihydrate	30% w/v Polyethylene glycol 8,000
C5	0.1 M HEPES sodium pH 7.5	None	0.8 M Potassium sodium tartrate tetrahydrate
C6	None	0.2 M Ammonium sulfate	30% w/v Polyethylene glycol 8,000
C7	None	0.2 M Ammonium sulfate	30% w/v Polyethylene glycol 4,000
C8	None	None	2.0 M Ammonium sulfate
C9	None	None	4.0 M Sodium formate
C10	0.1 M Sodium acetate trihydrate pH 4.6	None	2.0 M Sodium formate
C11	0.1 M HEPES sodium pH 7.5	None	0.8 M Sodium phosphate monobasic monohydrate,0.8 M Potassium phosphate monobasic
C12	0.1 M TRIS hydrochloride pH 8.5	None	8% w/v Polyethylene glycol 8,000
D1	0.1 M Sodium acetate trihydrate pH 4.6	None	8% w/v Polyethylene glycol 4,000
D2	0.1 M HEPES sodium pH 7.5	None	1.4 M Sodium citrate tribasic dihydrate
D3	0.1 M HEPES sodium pH 7.5	None	2% v/v Polyethylene glycol 400,2.0 M Ammonium sulfate
D4	0.1 M Sodium citrate tribasic dihydrate pH 5.6	None	20% v/v 2-Propanol,20% w/v Polyethylene glycol 4,000
D5	0.1 M HEPES sodium pH 7.5	None	10% v/v 2-Propanol,20% w/v Polyethylene glycol 4,000
D6	None	0.05 M Potassium phosphate monobasic	20% w/v Polyethylene glycol 8,000
D7	None	None	30% w/v Polyethylene glycol 1,500
D8	None	None	0.2 M Magnesium formate dihydrate
D9	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Zinc acetate dihydrate	18% w/v Polyethylene glycol 8,000
D10	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Calcium acetate hydrate	18% w/v Polyethylene glycol 8,000
D11	0.1 M Sodium acetate trihydrate pH 4.6	None	2.0 M Ammonium sulfate
D12	0.1 M TRIS hydrochloride pH 8.5	None	2.0 M Ammonium phosphate monobasic
E1	None	2.0 M Sodium chloride	10% w/v Polyethylene glycol 6,000
E2	None	0.5 M Sodium chloride,0.01 M Magnesium chloride hexahydrate	0.01 M Hexadecyltrimethylammonium bromide
E3	None	None	25% v/v Ethylene glycol
E4	None	None	35% v/v 1,4-Dioxane
E5	None	2.0 M Ammonium sulfate	5% v/v 2-Propanol
E6	None	None	1.0 M Imidazole pH 7.0
E7	None	None	10% w/v Polyethylene glycol 1,000,10% w/v Polyethylene glycol 8,000
E8	None	1.5 M Sodium chloride	10% v/v Ethanol
E9	0.1 M Sodium acetate trihydrate pH 4.6	None	2.0 M Sodium chloride
E10	0.1 M Sodium acetate trihydrate pH 4.6	0.2 M Sodium chloride	30% v/v (+/-)-2-Methyl-2,4-pentanediol
E11	0.1 M Sodium acetate trihydrate pH 4.6	0.01 M Cobalt(II) chloride hexahydrate	1.0 M 1,6-Hexanediol
E12	0.1 M Sodium acetate trihydrate pH 4.6	0.1 M Cadmium chloride hydrate	30% v/v Polyethylene glycol 400
F1	0.1 M Sodium acetate trihydrate pH 4.6	0.2 M Ammonium sulfate	30% w/v Polyethylene glycol monomethyl ether 2,000
F2	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.2 M Potassium sodium tartrate tetrahydrate	2.0 M Ammonium sulfate
F3	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.5 M Ammonium sulfate	1.0 M Lithium sulfate monohydrate
F4	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.5 M Sodium chloride	2% v/v Ethylene imine polymer
F5	0.1 M Sodium citrate tribasic dihydrate pH 5.6	None	35% v/v tert-Butanol
F6	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.01 M Iron(III) chloride hexahydrate	10% v/v Jeffamine ® M-600 ®

F7	0.1 M Sodium citrate tribasic dihydrate pH 5.6	None	2.5 M 1,6-Hexanediol
F8	0.1 M MES monohydrate pH 6.5	None	1.6 M Magnesium sulfate heptahydrate
F9	0.1 M MES monohydrate pH 6.5	0.1 M Sodium phosphate monobasic monohydrate,0.1 M Potassium phosphate monobasic	2.0 M Sodium chloride
F10	0.1 M MES monohydrate pH 6.5	None	12% w/v Polyethylene glycol 20,000
F11	0.1 M MES monohydrate pH 6.5	1.6 M Ammonium sulfate	10% v/v 1,4-Dioxane
F12	0.1 M MES monohydrate pH 6.5	0.05 M Cesium chloride	30% v/v Jeffamine ® M-600 ®
G1	0.1 M MES monohydrate pH 6.5	0.01 M Cobalt(II) chloride hexahydrate	1.8 M Ammonium sulfate
G2	0.1 M MES monohydrate pH 6.5	0.2 M Ammonium sulfate	30% w/v Polyethylene glycol monomethyl ether 5,000
G3	0.1 M MES monohydrate pH 6.5	0.01 M Zinc sulfate heptahydrate	25% v/v Polyethylene glycol monomethyl ether 550
G4	None	None	1.6 M Sodium citrate tribasic dihydrate pH 6.5
G5	0.1 M HEPES pH 7.5	0.5 M Ammonium sulfate	30% v/v (+/-)-2-Methyl-2,4-pentanediol
G6	0.1 M HEPES pH 7.5	None	10% w/v Polyethylene glycol 6,000,5% v/v (+/-)-2-Methyl-2,4-pentanediol
G7	0.1 M HEPES pH 7.5	None	20% v/v Jeffamine ® M-600 ®
G8	0.1 M HEPES pH 7.5	0.1 M Sodium chloride	1.6 M Ammonium sulfate
G9	0.1 M HEPES pH 7.5	None	2.0 M Ammonium formate
G10	0.1 M HEPES pH 7.5	0.05 M Cadmium sulfate hydrate	1.0 M Sodium acetate trihydrate
G11	0.1 M HEPES pH 7.5	None	70% v/v (+/-)-2-Methyl-2,4-pentanediol
G12	0.1 M HEPES pH 7.5	None	4.3 M Sodium chloride
H1	0.1 M HEPES pH 7.5	None	10% w/v Polyethylene glycol 8,000,8% v/v Ethylene glycol
H2	0.1 M HEPES pH 7.5	None	20% w/v Polyethylene glycol 10,000
H3	0.1 M Tris pH 8.5	0.2 M Magnesium chloride hexahydrate	3.4 M 1,6-Hexanediol
H4	0.1 M Tris pH 8.5	None	25% v/v tert-Butanol
H5	0.1 M Tris pH 8.5	0.01 M Nickel(II) chloride hexahydrate	1.0 M Lithium sulfate monohydrate
H6	0.1 M Tris pH 8.5	1.5 M Ammonium sulfate	12% v/v Glycerol
H7	0.1 M Tris pH 8.5	0.2 M Ammonium phosphate monobasic	50% v/v (+/-)-2-Methyl-2,4-pentanediol
H8	0.1 M Tris pH 8.5	None	20% v/v Ethanol
H9	0.1 M Tris pH 8.5	0.01 M Nickel(II) chloride hexahydrate	20% w/v Polyethylene glycol monomethyl ether 2,000
H10	0.1 M BICINE pH 9.0	0.1 M Sodium chloride	20% v/v Polyethylene glycol monomethyl ether 550
H11	0.1 M BICINE pH 9.0	None	2.0 M Magnesium chloride hexahydrate
H12	0.1 M BICINE pH 9.0	None	2% v/v 1,4-Dioxane,10% w/v Polyethylene glycol 20,000

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Well	Buffer	Salt
A1	0.1 M BIS-TRIS propane pH 7.0	1.8 M Sodium acetate trihydrate pH 7.0
A2	0.1 M BIS-TRIS propane pH 7.0	2.8 M Sodium acetate trihydrate pH 7.0
A3	0.1 M Sodium acetate trihydrate pH 4.6	1.5 M Ammonium chloride
A4	0.1 M BIS-TRIS propane pH 7.0	1.5 M Ammonium chloride
A5	0.1 M Tris pH 8.5	1.5 M Ammonium chloride
A6	0.1 M Sodium acetate trihydrate pH 4.6	3.5 M Ammonium chloride
A7	0.1 M BIS-TRIS propane pH 7.0	3.5 M Ammonium chloride
A8	0.1 M Tris pH 8.5	3.5 M Ammonium chloride
A9	0.1 M Sodium acetate trihydrate pH 4.6	2.2 M Sodium chloride
A10	0.1 M BIS-TRIS propane pH 7.0	2.2 M Sodium chloride
A11	0.1 M Tris pH 8.5	2.2 M Sodium chloride
A12	0.1 M Sodium acetate trihydrate pH 4.6	3.2 M Sodium chloride
B1	0.1 M BIS-TRIS propane pH 7.0	3.2 M Sodium chloride

B2	0.1 M Tris pH 8.5	3.2 M Sodium chloride
B3	0.1 M Sodium acetate trihydrate pH 4.6	1.0 M Ammonium citrate dibasic
B4	0.1 M Sodium acetate trihydrate pH 4.6	1.8 M Ammonium citrate dibasic
B5	0.1 M BIS-TRIS propane pH 7.0	1.0 M Ammonium citrate tribasic pH 7.0
B6	0.1 M BIS-TRIS propane pH 7.0	2.0 M Ammonium citrate tribasic pH 7.0
B7	0.1 M BIS-TRIS propane pH 7.0	0.7 M Sodium citrate tribasic dihydrate
B8	0.1 M Tris pH 8.5	0.7 M Sodium citrate tribasic dihydrate
B9	0.1 M BIS-TRIS propane pH 7.0	1.2 M Sodium citrate tribasic dihydrate
B10	0.1 M Tris pH 8.5	1.2 M Sodium citrate tribasic dihydrate
B11	0.1 M Sodium acetate trihydrate pH 4.6	0.4 M Magnesium formate dihydrate
B12	0.1 M BIS-TRIS propane pH 7.0	0.4 M Magnesium formate dihydrate
C1	0.1 M Tris pH 8.5	0.4 M Magnesium formate dihydrate
C2	0.1 M BIS-TRIS propane pH 7.0	0.7 M Magnesium formate dihydrate
C3	0.1 M Sodium acetate trihydrate pH 4.6	2.0 M Sodium formate
C4	0.1 M BIS-TRIS propane pH 7.0	2.0 M Sodium formate
C5	0.1 M Tris pH 8.5	2.0 M Sodium formate
C6	0.1 M Sodium acetate trihydrate pH 4.6	3.5 M Sodium formate
C7	0.1 M BIS-TRIS propane pH 7.0	3.5 M Sodium formate
C8	0.1 M Tris pH 8.5	3.5 M Sodium formate
C9	0.1 M BIS-TRIS propane pH 7.0	1.2 M DL-Malic acid pH 7.0
C10	0.1 M BIS-TRIS propane pH 7.0	2.2 M DL-Malic acid pH 7.0
C11	0.1 M BIS-TRIS propane pH 7.0	1.4 M Sodium malonate pH 7.0
C12	0.1 M BIS-TRIS propane pH 7.0	2.4 M Sodium malonate pH 7.0
D1	0.1 M Sodium acetate trihydrate pH 4.6	2.5 M Ammonium nitrate
D2	0.1 M BIS-TRIS propane pH 7.0	2.5 M Ammonium nitrate
D3	0.1 M Tris pH 8.5	2.5 M Ammonium nitrate
D4	0.1 M Sodium acetate trihydrate pH 4.6	6.0 M Ammonium nitrate
D5	0.1 M BIS-TRIS propane pH 7.0	6.0 M Ammonium nitrate
D6	0.1 M Tris pH 8.5	6.0 M Ammonium nitrate
D7	0.1 M Sodium acetate trihydrate pH 4.6	1.5 M Sodium nitrate
D8	0.1 M BIS-TRIS propane pH 7.0	1.5 M Sodium nitrate
D9	0.1 M Tris pH 8.5	1.5 M Sodium nitrate
D10	0.1 M Sodium acetate trihydrate pH 4.6	4.0 M Sodium nitrate
D11	0.1 M BIS-TRIS propane pH 7.0	4.0 M Sodium nitrate
D12	0.1 M Tris pH 8.5	4.0 M Sodium nitrate
E1	0.1 M Sodium acetate trihydrate pH 4.6	1.0 M Ammonium phosphate monobasic
E2	0.1 M Sodium acetate trihydrate pH 4.6	1.8 M Ammonium phosphate monobasic
E3	0.1 M Tris pH 8.5	1.5 M Ammonium phosphate dibasic
E4	0.1 M Tris pH 8.5	2.4 M Ammonium phosphate dibasic
E5	None	1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0
E6	None	1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9
E7	None	1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2
E8	None	1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0
E9	None	1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9
E10	None	1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2
E11	0.1 M BIS-TRIS propane pH 7.0	0.5 M Succinic acid pH 7.0
E12	0.1 M BIS-TRIS propane pH 7.0	1.0 M Succinic acid pH 7.0
F1	0.1 M Sodium acetate trihydrate pH 4.6	1.5 M Ammonium sulfate
F2	0.1 M BIS-TRIS propane pH 7.0	1.5 M Ammonium sulfate
F3	0.1 M Tris pH 8.5	1.5 M Ammonium sulfate

F4	0.1 M Sodium acetate trihydrate pH 4.6	2.5 M Ammonium sulfate
F5	0.1 M BIS-TRIS propane pH 7.0	2.5 M Ammonium sulfate
F6	0.1 M Tris pH 8.5	2.5 M Ammonium sulfate
F7	0.1 M Sodium acetate trihydrate pH 4.6	0.8 M Lithium sulfate monohydrate
F8	0.1 M BIS-TRIS propane pH 7.0	0.8 M Lithium sulfate monohydrate
F9	0.1 M Tris pH 8.5	0.8 M Lithium sulfate monohydrate
F10	0.1 M Sodium acetate trihydrate pH 4.6	1.5 M Lithium sulfate monohydrate
F11	0.1 M BIS-TRIS propane pH 7.0	1.5 M Lithium sulfate monohydrate
F12	0.1 M Tris pH 8.5	1.5 M Lithium sulfate monohydrate
G1	0.1 M Sodium acetate trihydrate pH 4.6	1.0 M Magnesium sulfate hydrate
G2	0.1 M BIS-TRIS propane pH 7.0	1.0 M Magnesium sulfate hydrate
G3	0.1 M Tris pH 8.5	1.0 M Magnesium sulfate hydrate
G4	0.1 M Sodium acetate trihydrate pH 4.6	1.8 M Magnesium sulfate hydrate
G5	0.1 M BIS-TRIS propane pH 7.0	1.8 M Magnesium sulfate hydrate
G6	0.1 M Tris pH 8.5	1.8 M Magnesium sulfate hydrate
G7	0.1 M Sodium acetate trihydrate pH 4.6	0.7 M Ammonium tartrate dibasic
G8	0.1 M BIS-TRIS propane pH 7.0	0.7 M Ammonium tartrate dibasic
G9	0.1 M Tris pH 8.5	0.7 M Ammonium tartrate dibasic
G10	0.1 M Sodium acetate trihydrate pH 4.6	1.0 M Ammonium tartrate dibasic
G11	0.1 M BIS-TRIS propane pH 7.0	1.3 M Ammonium tartrate dibasic
G12	0.1 M Tris pH 8.5	1.4 M Ammonium tartrate dibasic
H1	0.1 M BIS-TRIS propane pH 7.0	0.6 M Potassium sodium tartrate tetrahydrate
H2	0.1 M BIS-TRIS propane pH 7.0	1.2 M Potassium sodium tartrate tetrahydrate
H3	0.1 M Tris pH 8.5	0.6 M Potassium sodium tartrate tetrahydrate
H4	0.1 M Tris pH 8.5	1.2 M Potassium sodium tartrate tetrahydrate
H5	0.1 M Sodium acetate trihydrate pH 4.6	0.5 M Potassium thiocyanate
H6	0.1 M BIS-TRIS propane pH 7.0	0.5 M Potassium thiocyanate
H7	0.1 M Tris pH 8.5	0.5 M Potassium thiocyanate
H8	0.1 M Sodium acetate trihydrate pH 4.6	4.0 M Ammonium acetate
H9	0.1 M BIS-TRIS propane pH 7.0	4.0 M Ammonium acetate
H10	0.1 M Tris pH 8.5	4.0 M Ammonium acetate
H11	0.1 M BIS-TRIS propane pH 7.0	35% v/v Tacsimate pH 7.0
H12	0.1 M BIS-TRIS propane pH 7.0	60% v/v Tacsimate pH 7.0

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Well	Buffer	Salt	Precipitant
A1	0.1 M Sodium acetate trihydrate pH 4.6	0.1 M Sodium chloride	12% v/v (+/-)-2-Methyl-2,4-pentanediol
A2	0.1 M Sodium acetate trihydrate pH 4.6	0.1 M Zinc acetate dihydrate	12% w/v Polyethylene glycol 4,000
A3	0.1 M Sodium acetate trihydrate pH 4.6	0.2 M Ammonium sulfate	10% w/v Polyethylene glycol 4,000
A4	0.1 M Sodium acetate trihydrate pH 4.6	0.1 M Sodium chloride	12% v/v 2-Propanol
A5	0.1 M Sodium acetate trihydrate pH 4.6	None	12% w/v Polyethylene glycol 4,000
A6	0.1 M Sodium acetate trihydrate pH 4.6	None	1.0 M Ammonium sulfate
A7	0.1 M Sodium acetate trihydrate pH 4.6	None	1.0 M Magnesium sulfate heptahydrate
A8	0.1 M Sodium acetate trihydrate pH 4.6	0.1 M Magnesium chloride hexahydrate	18% v/v Polyethylene glycol 400
A9	0.1 M Sodium acetate trihydrate pH 4.6	0.1 M Lithium sulfate monohydrate	1.0 M Ammonium phosphate monobasic
A10	0.1 M Sodium acetate trihydrate pH 4.6	0.1 M Sodium chloride	12% w/v Polyethylene glycol 6,000
A11	0.1 M Sodium acetate trihydrate pH 4.6	0.1 M Magnesium chloride hexahydrate	12% w/v Polyethylene glycol 6,000
A12	0.1 M Sodium citrate tribasic	0.1 M Sodium chloride	18% v/v Polyethylene glycol 400

	dihydrate pH 5.6		
B1	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.1 M Lithium sulfate monohydrate	12% w/v Polyethylene glycol 4,000
B2	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.1 M Sodium citrate tribasic dihydrate	10% v/v 2-Propanol
B3	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.1 M Sodium chloride	12% v/v (+/-)-2-Methyl-2,4-pentanediol
B4	0.1 M Sodium citrate tribasic dihydrate pH 5.6	None	1.0 M Magnesium sulfate heptahydrate
B5	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.1 M Sodium chloride	12% w/v Polyethylene glycol 4,000
B6	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.1 M Lithium sulfate monohydrate	12% w/v Polyethylene glycol 6,000
B7	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.1 M Magnesium chloride hexahydrate	4% v/v (+/-)-2-Methyl-2,4-pentanediol
B8	0.1 M Sodium citrate tribasic dihydrate pH 5.6	None	0.1 M Sodium chloride
B9	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.1 M Lithium sulfate monohydrate	4% v/v Polyethylene glycol 400
B10	0.1 M ADA pH 6.5	None	1.0 M Ammonium sulfate
B11	0.1 M ADA pH 6.5	0.1 M Lithium sulfate monohydrate	12% w/v Polyethylene glycol 4,000, 2% v/v 2-Propanol
B12	0.1 M ADA pH 6.5	None	1.0 M Ammonium phosphate dibasic
C1	0.1 M ADA pH 6.5	0.1 M Magnesium chloride hexahydrate	12% w/v Polyethylene glycol 6,000
C2	0.1 M ADA pH 6.5	None	12% v/v (+/-)-2-Methyl-2,4-pentanediol
C3	0.1 M ADA pH 6.5	0.1 M Lithium sulfate monohydrate	1.0 M Magnesium sulfate hydrate
C4	0.1 M ADA pH 6.5	0.3 M Lithium sulfate monohydrate	4% v/v Polyethylene glycol 400
C5	0.1 M HEPES sodium pH 7.5	0.1 M Ammonium sulfate	0.5 M Sodium phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic
C6	0.1 M HEPES sodium pH 7.5	0.1 M Sodium chloride	10% w/v Polyethylene glycol 4,000
C7	0.1 M HEPES sodium pH 7.5	0.1 M Magnesium chloride hexahydrate	18% v/v Polyethylene glycol 400
C8	0.1 M HEPES sodium pH 7.5	None	1.0 M Potassium sodium tartrate tetrahydrate
C9	0.1 M HEPES sodium pH 7.5	0.1 M Ammonium sulfate	18% v/v Polyethylene glycol 400
C10	0.1 M HEPES sodium pH 7.5	0.1 M Ammonium sulfate	10% w/v Polyethylene glycol 4,000
C11	0.1 M HEPES sodium pH 7.5	0.1 M Sodium citrate tribasic dihydrate	12% v/v (+/-)-2-Methyl-2,4-pentanediol
C12	0.1 M HEPES sodium pH 7.5	None	1.0 M Sodium citrate tribasic dihydrate
D1	0.1 M HEPES sodium pH 7.5	0.6 M Magnesium sulfate hydrate	4% v/v Polyethylene glycol 400
D2	0.1 M HEPES sodium pH 7.5	0.6 M Magnesium sulfate hydrate	4% v/v (+/-)-2-Methyl-2,4-pentanediol
D3	0.1 M HEPES sodium pH 7.5	0.1 M Lithium sulfate monohydrate	0.1 M Potassium sodium tartrate tetrahydrate
D4	0.1 M TRIS hydrochloride pH 8.5	0.1 M Lithium sulfate monohydrate	12% v/v (+/-)-2-Methyl-2,4-pentanediol
D5	0.1 M TRIS hydrochloride pH 8.5	0.1 M Ammonium phosphate dibasic	0.5 M Sodium phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic
D6	0.1 M TRIS hydrochloride pH 8.5	None	0.1 M Sodium acetate trihydrate
D7	0.1 M TRIS hydrochloride pH 8.5	None	0.1 M Sodium chloride
D8	0.1 M TRIS hydrochloride pH 8.5	0.1 M Ammonium phosphate dibasic	12% w/v Polyethylene glycol 6,000
D9	0.1 M TRIS hydrochloride pH 8.5	0.1 M Potassium sodium tartrate tetrahydrate	0.4 M Magnesium sulfate hydrate
D10	0.1 M TRIS hydrochloride pH 8.5	None	0.2 M Lithium sulfate monohydrate
D11	0.1 M TRIS hydrochloride pH 8.5	None	0.5 M Ammonium sulfate
D12	0.1 M TRIS hydrochloride pH 8.5	0.1 M Sodium citrate tribasic dihydrate	5% v/v Polyethylene glycol 400
E1	0.1 M Sodium acetate trihydrate pH 4.6	0.02 M Calcium chloride dihydrate	15% v/v (+/-)-2-Methyl-2,4-pentanediol
E2	None	None	0.2 M Potassium sodium tartrate tetrahydrate
E3	None	None	0.2 M Ammonium phosphate monobasic
E4	0.1 M TRIS hydrochloride pH 8.5	None	1.0 M Ammonium sulfate
E5	0.1 M HEPES sodium pH 7.5	0.2 M Sodium citrate tribasic dihydrate	15% v/v (+/-)-2-Methyl-2,4-pentanediol
E6	0.1 M TRIS hydrochloride pH 8.5	0.2 M Magnesium chloride hexahydrate	15% w/v Polyethylene glycol 4,000
E7	0.1 M Sodium cacodylate trihydrate pH 6.5	None	0.7 M Sodium acetate trihydrate

E8	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Sodium citrate tribasic dihydrate	15% v/v 2-Propanol
E9	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.2 M Ammonium acetate	15% w/v Polyethylene glycol 4,000
E10	0.1 M Sodium acetate trihydrate pH 4.6	0.2 M Ammonium acetate	15% w/v Polyethylene glycol 4,000
E11	0.1 M Sodium citrate tribasic dihydrate pH 5.6	None	0.5 M Ammonium phosphate monobasic
E12	0.1 M HEPES sodium pH 7.5	0.2 M Magnesium chloride hexahydrate	15% v/v 2-Propanol
F1	0.1 M TRIS hydrochloride pH 8.5	0.2 M Sodium citrate tribasic dihydrate	15% v/v Polyethylene glycol 400
F2	0.1 M HEPES sodium pH 7.5	0.2 M Calcium chloride dihydrate	14% v/v Polyethylene glycol 400
F3	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Ammonium sulfate	15% w/v Polyethylene glycol 8,000
F4	0.1 M HEPES sodium pH 7.5	None	0.75 M Lithium sulfate monohydrate
F5	0.1 M TRIS hydrochloride pH 8.5	0.2 M Lithium sulfate monohydrate	15% w/v Polyethylene glycol 4,000
F6	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Magnesium acetate tetrahydrate	10% w/v Polyethylene glycol 8,000
F7	0.1 M TRIS hydrochloride pH 8.5	0.2 M Ammonium acetate	15% v/v 2-Propanol
F8	0.1 M Sodium acetate trihydrate pH 4.6	0.2 M Ammonium sulfate	12.5% w/v Polyethylene glycol 4,000
F9	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Magnesium acetate tetrahydrate	15% v/v (+/-)-2-Methyl-2,4-pentanediol
F10	0.1 M TRIS hydrochloride pH 8.5	0.2 M Sodium acetate trihydrate	15% w/v Polyethylene glycol 4,000
F11	0.1 M HEPES sodium pH 7.5	0.2 M Magnesium chloride hexahydrate	15% v/v Polyethylene glycol 400
F12	0.1 M Sodium acetate trihydrate pH 4.6	0.2 M Calcium chloride dihydrate	10% v/v 2-Propanol
G1	0.1 M Imidazole pH 6.5	None	0.5 M Sodium acetate trihydrate
G2	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.2 M Ammonium acetate	15% v/v (+/-)-2-Methyl-2,4-pentanediol
G3	0.1 M HEPES sodium pH 7.5	0.2 M Sodium citrate tribasic dihydrate	10% v/v 2-Propanol
G4	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Sodium acetate trihydrate	15% w/v Polyethylene glycol 8,000
G5	0.1 M HEPES sodium pH 7.5	None	0.4 M Potassium sodium tartrate tetrahydrate
G6	None	0.2 M Ammonium sulfate	15% w/v Polyethylene glycol 8,000
G7	None	0.2 M Ammonium sulfate	15% w/v Polyethylene glycol 4,000
G8	None	None	1.0 M Ammonium sulfate
G9	None	None	2.0 M Sodium formate
G10	0.1 M Sodium acetate trihydrate pH 4.6	None	1.0 M Sodium formate
G11	0.1 M HEPES sodium pH 7.5	None	0.4 M Sodium phosphate monobasic monohydrate, 0.4 M Potassium phosphate monobasic
G12	0.1 M TRIS hydrochloride pH 8.5	None	4% w/v Polyethylene glycol 8,000
H1	0.1 M Sodium acetate trihydrate pH 4.6	None	4% w/v Polyethylene glycol 4,000
H2	0.1 M HEPES sodium pH 7.5	None	0.7 M Sodium citrate tribasic dihydrate
H3	0.1 M HEPES sodium pH 7.5	None	2% v/v Polyethylene glycol 400, 1.0 M Ammonium sulfate
H4	0.1 M Sodium citrate tribasic dihydrate pH 5.6	None	10% v/v 2-Propanol, 10% w/v Polyethylene glycol 4,000
H5	0.1 M HEPES sodium pH 7.5	None	5% v/v 2-Propanol, 10% w/v Polyethylene glycol 4,000
H6	None	0.05 M Potassium phosphate monobasic	10% w/v Polyethylene glycol 8,000
H7	None	None	15% w/v Polyethylene glycol 1,500
H8	None	None	0.1 M Magnesium formate dihydrate
H9	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Zinc acetate dihydrate	9% w/v Polyethylene glycol 8,000
H10	0.1 M Sodium cacodylate trihydrate pH 6.5	0.2 M Calcium acetate hydrate	9% w/v Polyethylene glycol 8,000
H11	0.1 M Sodium acetate trihydrate pH 4.6	None	1.0 M Ammonium sulfate
H12	0.1 M TRIS hydrochloride pH 8.5	None	1.0 M Ammonium phosphate monobasic

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Well	Salt	Buffer	Polymer	pH
A1	0.2 M Sodium fluoride		20% w/v Polyethylene glycol 3,350	7.3
A2	0.2 M Potassium fluoride		20% w/v Polyethylene glycol 3,350	7.3
A3	0.2 M Ammonium fluoride		20% w/v Polyethylene glycol 3,350	6.2
A4	0.2 M Lithium chloride		20% w/v Polyethylene glycol 3,350	6.8
A5	0.2 M Magnesium chloride hexahydrate		20% w/v Polyethylene glycol 3,350	5.9
A6	0.2 M Sodium chloride		20% w/v Polyethylene glycol 3,350	6.9
A7	0.2 M Calcium chloride dihydrate		20% w/v Polyethylene glycol 3,350	5.1
A8	0.2 M Potassium chloride		20% w/v Polyethylene glycol 3,350	7.0
A9	0.2 M Ammonium chloride		20% w/v Polyethylene glycol 3,350	6.3
A10	0.2 M Sodium iodide		20% w/v Polyethylene glycol 3,350	7.0
A11	0.2 M Potassium iodide		20% w/v Polyethylene glycol 3,350	7.0
A12	0.2 M Ammonium iodide		20% w/v Polyethylene glycol 3,350	6.2
B1	0.2 M Sodium thiocyanate		20% w/v Polyethylene glycol 3,350	6.9
B2	0.2 M Potassium thiocyanate		20% w/v Polyethylene glycol 3,350	7.0
B3	0.2 M Lithium nitrate		20% w/v Polyethylene glycol 3,350	7.1
B4	0.2 M Magnesium nitrate hexahydrate		20% w/v Polyethylene glycol 3,350	5.9
B5	0.2 M Sodium nitrate		20% w/v Polyethylene glycol 3,350	6.8
B6	0.2 M Potassium nitrate		20% w/v Polyethylene glycol 3,350	6.8
B7	0.2 M Ammonium nitrate		20% w/v Polyethylene glycol 3,350	6.2
B8	0.2 M Magnesium formate dihydrate		20% w/v Polyethylene glycol 3,350	7.0
B9	0.2 M Sodium formate		20% w/v Polyethylene glycol 3,350	7.2
B10	0.2 M Potassium formate		20% w/v Polyethylene glycol 3,350	7.3
B11	0.2 M Ammonium formate		20% w/v Polyethylene glycol 3,350	6.6
B12	0.2 M Lithium acetate dihydrate		20% w/v Polyethylene glycol 3,350	7.9
C1	0.2 M Magnesium acetate tetrahydrate		20% w/v Polyethylene glycol 3,350	7.9
C2	0.2 M Zinc acetate dihydrate		20% w/v Polyethylene glycol 3,350	6.4
C3	0.2 M Sodium acetate trihydrate		20% w/v Polyethylene glycol 3,350	8.0
C4	0.2 M Calcium acetate hydrate		20% w/v Polyethylene glycol 3,350	7.5
C5	0.2 M Potassium acetate		20% w/v Polyethylene glycol 3,350	8.1
C6	0.2 M Ammonium acetate		20% w/v Polyethylene glycol 3,350	7.1
C7	0.2 M Lithium sulfate monohydrate		20% w/v Polyethylene glycol 3,350	6.0
C8	0.2 M Magnesium sulfate heptahydrate		20% w/v Polyethylene glycol 3,350	6.0
C9	0.2 M Sodium sulfate decahydrate		20% w/v Polyethylene glycol 3,350	6.7
C10	0.2 M Potassium sulfate		20% w/v Polyethylene glycol 3,350	6.8
C11	0.2 M Ammonium sulfate		20% w/v Polyethylene glycol 3,350	6.0
C12	0.2 M Sodium tartrate dibasic dihydrate		20% w/v Polyethylene glycol 3,350	7.3
D1	0.2 M Potassium sodium tartrate tetrahydrate		20% w/v Polyethylene glycol 3,350	7.4
D2	0.2 M Ammonium tartrate dibasic		20% w/v Polyethylene glycol 3,350	6.6
D3	0.2 M Sodium phosphate monobasic monohydrate		20% w/v Polyethylene glycol 3,350	4.7
D4	0.2 M Sodium phosphate dibasic dihydrate		20% w/v Polyethylene glycol 3,350	9.1
D5	0.2 M Potassium phosphate monobasic		20% w/v Polyethylene glycol 3,350	4.8
D6	0.2 M Potassium phosphate dibasic		20% w/v Polyethylene glycol 3,350	9.2
D7	0.2 M Ammonium phosphate monobasic		20% w/v Polyethylene glycol 3,350	4.6
D8	0.2 M Ammonium phosphate		20% w/v Polyethylene glycol 3,350	8.0

	dibasic			
D9	0.2 M Lithium citrate tribasic tetrahydrate		20% w/v Polyethylene glycol 3,350	8.4
D10	0.2 M Sodium citrate tribasic dihydrate		20% w/v Polyethylene glycol 3,350	8.3
D11	0.2 M Potassium citrate tribasic monohydrate		20% w/v Polyethylene glycol 3,350	8.3
D12	0.2 M Ammonium citrate dibasic		20% w/v Polyethylene glycol 3,350	5.1
E1	0.1 M Sodium malonate pH 4.0	None	12% w/v Polyethylene glycol 3,350	
E2	0.2 M Sodium malonate pH 4.0	None	20% w/v Polyethylene glycol 3,350	
E3	0.1 M Sodium malonate pH 5.0	None	12% w/v Polyethylene glycol 3,350	
E4	0.2 M Sodium malonate pH 5.0	None	20% w/v Polyethylene glycol 3,350	
E5	0.1 M Sodium malonate pH 6.0	None	12% w/v Polyethylene glycol 3,350	
E6	0.2 M Sodium malonate pH 6.0	None	20% w/v Polyethylene glycol 3,350	
E7	0.1 M Sodium malonate pH 7.0	None	12% w/v Polyethylene glycol 3,350	
E8	0.2 M Sodium malonate pH 7.0	None	20% w/v Polyethylene glycol 3,350	
E9	4% v/v Tacsimate pH 4.0	None	12% w/v Polyethylene glycol 3,350	
E10	8% v/v Tacsimate pH 4.0	None	20% w/v Polyethylene glycol 3,350	
E11	4% v/v Tacsimate pH 5.0	None	12% w/v Polyethylene glycol 3,350	
E12	8% v/v Tacsimate pH 5.0	None	20% w/v Polyethylene glycol 3,350	
F1	4% v/v Tacsimate pH 6.0	None	12% w/v Polyethylene glycol 3,350	
F2	8% v/v Tacsimate pH 6.0	None	20% w/v Polyethylene glycol 3,350	
F3	4% v/v Tacsimate pH 7.0	None	12% w/v Polyethylene glycol 3,350	
F4	8% v/v Tacsimate pH 7.0	None	20% w/v Polyethylene glycol 3,350	
F5	4% v/v Tacsimate pH 8.0	None	12% w/v Polyethylene glycol 3,350	
F6	8% v/v Tacsimate pH 8.0	None	20% w/v Polyethylene glycol 3,350	
F7	0.1 M Succinic acid pH 7.0	None	12% w/v Polyethylene glycol 3,350	
F8	0.2 M Succinic acid pH 7.0	None	20% w/v Polyethylene glycol 3,350	
F9	0.1 M Ammonium citrate tribasic pH 7.0	None	12% w/v Polyethylene glycol 3,350	
F10	0.2 M Ammonium citrate tribasic pH 7.0	None	20% w/v Polyethylene glycol 3,350	
F11	0.1 M DL-Malic acid pH 7.0	None	12% w/v Polyethylene glycol 3,350	
F12	0.2 M DL-Malic acid pH 7.0	None	20% w/v Polyethylene glycol 3,350	
G1	0.1 M Sodium acetate trihydrate pH 7.0	None	12% w/v Polyethylene glycol 3,350	
G2	0.2 M Sodium acetate trihydrate pH 7.0	None	20% w/v Polyethylene glycol 3,350	
G3	0.1 M Sodium formate pH 7.0	None	12% w/v Polyethylene glycol 3,350	
G4	0.2 M Sodium formate pH 7.0	None	20% w/v Polyethylene glycol 3,350	
G5	0.1 M Ammonium tartrate dibasic pH 7.0	None	12% w/v Polyethylene glycol 3,350	
G6	0.2 M Ammonium tartrate dibasic pH 7.0	None	20% w/v Polyethylene glycol 3,350	
G7	2% v/v Tacsimate pH 4.0	0.1 M Sodium acetate trihydrate pH 4.6	16% w/v Polyethylene glycol 3,350	
G8	2% v/v Tacsimate pH 5.0	0.1 M Sodium citrate tribasic dihydrate pH 5.6	16% w/v Polyethylene glycol 3,350	
G9	2% v/v Tacsimate pH 6.0	0.1 M BIS-TRIS pH 6.5	20% w/v Polyethylene glycol 3,350	
G10	2% v/v Tacsimate pH 7.0	0.1 M HEPES pH 7.5	20% w/v Polyethylene glycol 3,350	
G11	2% v/v Tacsimate pH 8.0	0.1 M Tris pH 8.5	16% w/v Polyethylene glycol 3,350	
G12	None	0.07 M Citric acid, 0.03 M BIS-TRIS propane / pH 3.4	16% w/v Polyethylene glycol 3,350	
H1	None	0.06 M Citric acid, 0.04 M BIS-TRIS propane / pH 4.1	16% w/v Polyethylene glycol 3,350	
H2	None	0.05 M Citric acid, 0.05 M BIS-TRIS propane / pH 5.0	16% w/v Polyethylene glycol 3,350	
H3	None	0.04 M Citric acid, 0.06 M BIS-TRIS propane / pH 6.4	20% w/v Polyethylene glycol 3,350	
H4	None	0.03 M Citric acid, 0.07 M BIS-TRIS propane / pH 7.6	20% w/v Polyethylene glycol 3,350	
H5	None	0.02 M Citric acid, 0.08 M BIS-TRIS propane / pH 8.8	16% w/v Polyethylene glycol 3,350	

H6	0.02 M Calcium chloride dihydrate, 0.02 M Cadmium chloride hydrate, 0.02 M Cobalt(II) chloride hexahydrate	None	20% w/v Polyethylene glycol 3,350	
H7	0.01 M Magnesium chloride hexahydrate,0.005 M Nickel(II) chloride hexahydrate	0.1 M HEPES sodium pH 7.0	15% w/v Polyethylene glycol 3,350	
H8	0.02 M Zinc chloride	None	20% w/v Polyethylene glycol 3,350	
H9	0.15 M Cesium chloride	None	15% w/v Polyethylene glycol 3,350	
H10	0.2 M Sodium bromide	None	20% w/v Polyethylene glycol 3,350	
H11	1% w/v Tryptone	0.05 M HEPES sodium pH 7.0	12% w/v Polyethylene glycol 3,350	
H12	1% w/v Tryptone	0.05 M HEPES sodium pH 7.0	20% w/v Polyethylene glycol 3,350	

Structure Screen 1 & 2

Well	Salt	Buffer	pH	Precipitant
A1	0.02 M calcium chloride	0.1 M Na acetate	4.6	30 % v/v MPD
A2	0.2 M ammonium acetate	0.1 M Na acetate	4.6	30 % w/v PEG 4000
A3	0.2 M ammonium sulfate	0.1 M Na acetate	4.6	25 % w/v PEG 4000
A4	None	0.1 M Na acetate	4.6	2.0 M sodium formate
A5	None	0.1 M Na acetate	4.6	2.0 M ammonium sulfate
A6	None	0.1 M Na acetate	4.6	8 % w/v PEG 4000
A7	0.2 M ammonium acetate	0.1 M Na citrate	5.6	30 % w/v PEG 4000
A8	0.2 M ammonium acetate	0.1 M Na citrate	5.6	30 % v/v MPD
A9	None	0.1 M Na citrate	5.6	20 % v/v 2-propanol, 20 % w/v PEG 4000
A10	None	0.1 M Na citrate	5.6	1.0 M ammonium dihydrogen phosphate
A11	0.2 M calcium chloride	0.1 M Na acetate	4.6	20 % v/v 2-propanol
A12	None	0.1 M Na cacodylate	6.5	1.4 M sodium acetate
B1	0.2 M sodium citrate	0.1 M Na cacodylate	6.5	30 % v/v 2-propanol
B2	0.2 M ammonium sulfate	0.1 M Na cacodylate	6.5	30 % w/v PEG 8000
B3	0.2 M magnesium acetate	0.1 M Na cacodylate	6.5	20 % w/v PEG 8000
B4	0.2 M magnesium acetate	0.1 M Na cacodylate	6.5	30 % v/v MPD
B5	None	0.1 M imidazole	6.5	1.0 M sodium acetate
B6	0.2 M sodium acetate	0.1 M Na cacodylate	6.5	30 % w/v PEG 8000
B7	0.2 M zinc acetate	0.1 M Na cacodylate	6.5	18 % w/v PEG 8000
B8	0.2 M calcium acetate	0.1 M Na cacodylate	6.5	18 % w/v PEG 8000
B9	0.2 M sodium citrate	0.1 M Na HEPES	7.5	30 % v/v MPD
B10	0.2 M magnesium chloride	0.1 M Na HEPES	7.5	30 % v/v 2-propanol
B11	0.2 M calcium chloride	0.1 M Na HEPES	7.5	28 % v/v PEG 400
B12	0.2 M magnesium chloride	0.1 M Na HEPES	7.5	30 % v/v PEG 400
C1	0.2 M sodium citrate	0.1 M Na HEPES	7.5	20 % v/v 2-propanol
C2	None	0.1 M Na HEPES	7.5	0.8 M K/Na tartrate
C3	None	0.1 M Na HEPES	7.5	1.5 M lithium sulfate
C4	None	0.1 M Na HEPES	7.5	0.8 M sodium dihydrogen phosphate, 0.8 M potassium dihydrogen phosphate
C5	None	0.1 M Na HEPES	7.5	1.4 M tri-sodium citrate
C6	None	0.1 M Na HEPES	7.5	2 % v/v PEG 400,2.0 M ammonium sulfate
C7	None	0.1 M Na HEPES	7.5	10 % v/v 2-propanol, 20 % w/v PEG 4000
C8	None	0.1 M Tris	8.5	2.0 M ammonium sulfate
C9	0.2 M magnesium chloride	0.1 M Tris	8.5	30 % w/v PEG 4000
C10	0.2 M sodium citrate	0.1 M Tris	8.5	30 % v/v PEG 400
C11	0.2 M lithium sulfate	0.1 M Tris	8.5	30 % w/v PEG 4000
C12	0.2 M ammonium acetate	0.1 M Tris	8.5	30 % v/v 2-propanol
D1	0.2 M sodium acetate	0.1 M Tris	8.5	30 % w/v PEG 4000
D2	None	0.1 M Tris	8.5	8 % w/v PEG 8000
D3	None	0.1 M Tris	8.5	2.0 M ammonium dihydrogen phosphate

D4	None	None	None	0.4 M K/Na tartrate
D5	None	None	None	0.4 M ammonium dihydrogen phosphate
D6	0.2 M ammonium sulfate	None	None	30 % w/v PEG 8000
D7	0.2 M ammonium sulfate	None	None	30 % w/v PEG 4000
D8	None	None	None	2.0 M ammonium sulfate
D9	None	None	None	4.0 M sodium formate
D10	0.05 M potassium dihydrogen phosphate	None	None	20 % w/v PEG 8000
D11	None	None	None	30 % w/v PEG 1500
D12	None	None	None	0.2 M magnesium formate
E1	0.1 M sodium chloride	0.1 M Bicine	9.0	30 % v/v PEG 550 MME
E2	None	0.1 M Bicine	9.0	2.0 M magnesium chloride
E3	2 % v/v dioxane	0.1 M Bicine	9.0	10 % w/v PEG 20,000
E4	0.2 M magnesium chloride	0.1 M Tris	8.5	3.4 M 1,6-hexanediol
E5	None	0.1 M Tris	8.5	25 % v/v tert-Butanol
E6	0.01 M nickel chloride	0.1 M Tris	8.5	1.0 M lithium sulfate
E7	1.5 M ammonium sulfate	0.1 M Tris	8.5	12 % v/v glycerol
E8	0.2 M ammonium phosphate monobasic	0.1 M Tris	8.5	50 % v/v MPD
E9	None	0.1 M Tris	8.5	20 % v/v ethanol
E10	0.01 M nickel chloride	0.1 M Tris	8.5	20 % w/v PEG 2000 MME
E11	0.5 M ammonium sulfate	0.1 M Na HEPES	8.5	30 % v/v MPD
E12	None	0.1 M Na HEPES	7.5	10 % w/v PEG 6000, 5% v/v MPD
F1	None	0.1 M Na HEPES	7.5	20 % v/v Jeffamine M-600
F2	0.1 M sodium chloride	0.1 M Na HEPES	7.5	1.6 M ammonium sulfate
F3	None	0.1 M Na HEPES	7.5	2.0 M ammonium formate
F4	0.05 M cadmium sulfate	0.1 M Na HEPES	7.5	1.0 M sodium acetate
F5	None	0.1 M Na HEPES	7.5	70 % v/v MPD
F6	None	0.1 M Na HEPES	7.5	4.3 M sodium chloride
F7	None	0.1 M Na HEPES	7.5	10 % w/v PEG 8000, 8 % v/v ethylene glycol
F8	None	0.1 M MES	6.5	1.6 M magnesium sulfate
F9	0.1 M sodium dihydrogen phosphate, 0.1 M potassium dihydrogen phosphate	0.1 M MES	6.5	2.0 M sodium chloride
F10	None	0.1 M MES	6.5	12 % w/v PEG 20,000
F11	1.6 M ammonium sulfate	0.1 M MES	6.5	10 % v/v dioxane
F12	0.05 M caesium chloride	0.1 M MES	6.5	30 % v/v Jeffamine M-600
G1	0.01 M cobalt chloride	0.1 M MES	6.5	1.8 M ammonium sulfate
G2	0.2 M ammonium sulfate	0.1 M MES	6.5	30 % w/v PEG 5000 MME
G3	0.01 M zinc sulfate	0.1 M MES	6.5	25 % v/v PEG 550 MME
G4	0.1 M Na HEPES	0.1 M Na HEPES	7.5	20 % w/v PEG 10,000
G5	0.2 M K/Na Tartrate	0.1 M Na citrate	5.6	2.0 M ammonium sulfate
G6	0.5 M ammonium sulfate	0.1 M Na citrate	5.6	1.0 M lithium sulfate
G7	0.5 M sodium chloride	0.1 M Na citrate	5.6	4% v/v polyethyleneimine
G8	None	0.1 M Na citrate	5.6	35 % v/v tert-Butanol
G9	0.01 M ferric chloride	0.1 M Na citrate	5.6	10 % v/v Jeffamine M-600
G10	0.01 M manganese chloride	0.1 M Na citrate	5.6	2.5 M 1,6-hexanediol
G11	None	0.1 M Na acetate	4.6	2.0 M sodium chloride
G12	0.2 M sodium chloride	0.1 M Na acetate	4.7	30 % v/v MPD
H1	0.01 M cobalt chloride	0.1 M Na acetate	4.8	1.0 M 1,6-hexanediol
H2	0.1 M cadmium chloride	0.1 M Na acetate	4.9	30 % v/v PEG 400
H3	0.2 M ammonium sulfate	0.1 M Na acetate	4.10	30 % w/v PEG 2000 MME
H4	2.0 M sodium chloride	None	None	10 % w/v PEG 6000
H5	0.01 M CTAB	None	None	0.5 M sodium chloride,0.1 M magnesium chloride
H6	None	None	None	25 % v/v ethylene glycol
H7	None	None	None	35 % v/v dioxane

H8	2.0 M ammonium sulfate	None	None	5 % v/v 2-propanol
H9	None	None	7.0	1.0 M imidazole
H10	None	None	None	10 % w/v PEG 1000, 10% w/v PEG 8000
H11	1.5 M sodium chloride	None	None	10 % v/v ethanol
H12	None	None	6.5	1.6 M sodium citrate

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Well	Salt	Buffer	Precipitant
A1	None	0.1 M Citric acid pH 3.5	2.0 M Ammonium sulfate
A2	None	0.1 M Sodium acetate trihydrate pH 4.5	2.0 M Ammonium sulfate
A3	None	0.1 M BIS-TRIS pH 5.5	2.0 M Ammonium sulfate
A4	None	0.1 M BIS-TRIS pH 6.5	2.0 M Ammonium sulfate
A5	None	0.1 M HEPES pH 7.5	2.0 M Ammonium sulfate
A6	None	0.1 M Tris pH 8.5	2.0 M Ammonium sulfate
A7	None	0.1 M Citric acid pH 3.5	3.0 M Sodium chloride
A8	None	0.1 M Sodium acetate trihydrate pH 4.5	3.0 M Sodium chloride
A9	None	0.1 M BIS-TRIS pH 5.5	3.0 M Sodium chloride
A10	None	0.1 M BIS-TRIS pH 6.5	3.0 M Sodium chloride
A11	None	0.1 M HEPES pH 7.5	3.0 M Sodium chloride
A12	None	0.1 M Tris pH 8.5	3.0 M Sodium chloride
B1	None	0.1 M BIS-TRIS pH 5.5	0.3 M Magnesium formate dihydrate
B2	None	0.1 M BIS-TRIS pH 6.5	0.5 M Magnesium formate dihydrate
B3	None	0.1 M HEPES pH 7.5	0.5 M Magnesium formate dihydrate
B4	None	0.1 M Tris pH 8.5	0.3 M Magnesium formate dihydrate
B5	None	None - pH 5.6	1.26 M Sodium phosphate monobasic monohydrate, 0.14 M Potassium phosphate dibasic
B6	None	None - pH 6.9	0.49 M Sodium phosphate monobasic monohydrate, 0.91 M Potassium phosphate dibasic
B7	None	None - pH 8.2	0.056 M Sodium phosphate monobasic monohydrate, 1.344 M Potassium phosphate dibasic
B8	None	0.1 M HEPES pH 7.5	1.4 M Sodium citrate tribasic dihydrate
B9	None	None	1.8 M Ammonium citrate tribasic pH 7.0
B10	None	None	0.8 M Succinic acid pH 7.0
B11	None	None	2.1 M DL-Malic acid pH 7.0
B12	None	None	2.8 M Sodium acetate trihydrate pH 7.0
C1	None	None	3.5 M Sodium formate pH 7.0
C2	None	None	1.1 M Ammonium tartrate dibasic pH 7.0
C3	None	None	2.4 M Sodium malonate pH 7.0
C4	None	None	35% v/v Tacsimate pH 7.0
C5	None	None	60% v/v Tacsimate pH 7.0
C6	0.1 M Sodium chloride	0.1 M BIS-TRIS pH 6.5	1.5 M Ammonium sulfate
C7	0.8 M Potassium sodium tartrate tetrahydrate	0.1 M Tris pH 8.5	0.5% w/v Polyethylene glycol monomethyl ether 5,000
C8	1.0 M Ammonium sulfate	0.1 M BIS-TRIS pH 5.5	1% w/v Polyethylene glycol 3,350
C9	1.1 M Sodium malonate pH 7.0	0.1 M HEPES pH 7.0	0.5% v/v Jeffamine ® ED-2001 pH 7.0
C10	1.0 M Succinic acid pH 7.0	0.1 M HEPES pH 7.0	1% w/v Polyethylene glycol monomethyl ether 2,000
C11	1.0 M Ammonium sulfate	0.1 M HEPES pH 7.0	0.5% w/v Polyethylene glycol 8,000
C12	15% v/v Tacsimate pH 7.0	0.1 M HEPES pH 7.0	2% w/v Polyethylene glycol 3,350
D1	None	None	25% w/v Polyethylene glycol 1,500
D2	None	0.1 M HEPES pH 7.0	30% v/v Jeffamine ® M-600 ® pH 7.0
D3	None	0.1 M HEPES pH 7.0	30% v/v Jeffamine ® ED-2001 pH 7.0

D4	None	0.1 M Citric acid pH 3.5	25% w/v Polyethylene glycol 3,350
D5	None	0.1 M Sodium acetate trihydrate pH 4.5	25% w/v Polyethylene glycol 3,350
D6	None	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
D7	None	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
D8	None	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
D9	None	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
D10	None	0.1 M BIS-TRIS pH 6.5	20% w/v Polyethylene glycol monomethyl ether 5,000
D11	None	0.1 M BIS-TRIS pH 6.5	28% w/v Polyethylene glycol monomethyl ether 2,000
D12	0.2 M Calcium chloride dihydrate	0.1 M BIS-TRIS pH 5.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
E1	0.2 M Calcium chloride dihydrate	0.1 M BIS-TRIS pH 6.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
E2	0.2 M Ammonium acetate	0.1 M BIS-TRIS pH 5.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
E3	0.2 M Ammonium acetate	0.1 M BIS-TRIS pH 6.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
E4	0.2 M Ammonium acetate	0.1 M HEPES pH 7.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
E5	0.2 M Ammonium acetate	0.1 M Tris pH 8.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
E6	0.05 M Calcium chloride dihydrate	0.1 M BIS-TRIS pH 6.5	30% v/v Polyethylene glycol monomethyl ether 550
E7	0.05 M Magnesium chloride hexahydrate	0.1 M HEPES pH 7.5	30% v/v Polyethylene glycol monomethyl ether 550
E8	0.2 M Potassium chloride	0.05 M HEPES pH 7.5	35% v/v Pentaerythritol propoxylate (5/4 PO/OH)
E9	0.05 M Ammonium sulfate	0.05 M BIS-TRIS pH 6.5	30% v/v Pentaerythritol ethoxylate (15/4 EO/OH)
E10	None	0.1 M BIS-TRIS pH 6.5	45% v/v Polypropylene glycol P 400
E11	0.02 M Magnesium chloride hexahydrate	0.1 M HEPES pH 7.5	22% w/v Poly(acrylic acid sodium salt) 5,100
E12	0.01 M Cobalt(II) chloride hexahydrate	0.1 M Tris pH 8.5	20% w/v Polyvinylpyrrolidone K 15
F1	0.2 M L-Proline	0.1 M HEPES pH 7.5	10% w/v Polyethylene glycol 3,350
F2	0.2 M Trimethylamine N-oxide dihydrate	0.1 M Tris pH 8.5	20% w/v Polyethylene glycol monomethyl ether 2,000
F3	5% v/v Tacsimate pH 7.0	0.1 M HEPES pH 7.0	10% w/v Polyethylene glycol monomethyl ether 5,000
F4	0.005 M Cobalt(II) chloride hexahydrate, 0.005 M Nickel(II) chloride hexahydrate, 0.005 M Cadmium chloride hydrate, 0.005 M Magnesium chloride hexahydrate	0.1 M HEPES pH 7.5	12% w/v Polyethylene glycol 3,350
F5	0.1 M Ammonium acetate	0.1 M BIS-TRIS pH 5.5	17% w/v Polyethylene glycol 10,000
F6	0.2 M Ammonium sulfate	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
F7	0.2 M Ammonium sulfate	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
F8	0.2 M Ammonium sulfate	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
F9	0.2 M Ammonium sulfate	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
F10	0.2 M Sodium chloride	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
F11	0.2 M Sodium chloride	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
F12	0.2 M Sodium chloride	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
G1	0.2 M Sodium chloride	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
G2	0.2 M Lithium sulfate monohydrate	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
G3	0.2 M Lithium sulfate monohydrate	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
G4	0.2 M Lithium sulfate monohydrate	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
G5	0.2 M Lithium sulfate monohydrate	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
G6	0.2 M Ammonium acetate	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
G7	0.2 M Ammonium acetate	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
G8	0.2 M Ammonium acetate	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
G9	0.2 M Ammonium acetate	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
G10	0.2 M Magnesium chloride hexahydrate	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350

G11	0.2 M Magnesium chloride hexahydrate	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
G12	0.2 M Magnesium chloride hexahydrate	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
H1	0.2 M Magnesium chloride hexahydrate	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
H2	0.2 M Potassium sodium tartrate tetrahydrate	None	20% w/v Polyethylene glycol 3,350
H3	0.2 M Sodium malonate pH 7.0	None	20% w/v Polyethylene glycol 3,350
H4	0.2 M Ammonium citrate tribasic pH 7.0	None	20% w/v Polyethylene glycol 3,350
H5	0.1 M Succinic acid pH 7.0	None	15% w/v Polyethylene glycol 3,350
H6	0.2 M Sodium formate	None	20% w/v Polyethylene glycol 3,350
H7	0.15 M DL-Malic acid pH 7.0	None	20% w/v Polyethylene glycol 3,350
H8	0.1 M Magnesium formate dihydrate	None	15% w/v Polyethylene glycol 3,350
H9	0.05 M Zinc acetate dihydrate	None	20% w/v Polyethylene glycol 3,350
H10	0.2 M Sodium citrate tribasic dihydrate	None	20% w/v Polyethylene glycol 3,350
H11	0.1 M Potassium thiocyanate	None	30% w/v Polyethylene glycol monomethyl ether 2,000
H12	0.15 M Potassium bromide	None	30% w/v Polyethylene glycol monomethyl ether 2,000

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Well	Salt	Buffer	Precipitant	Glycerol
A1	0.02 M Calcium chloride dihydrate	0.1 M Sodium acetate trihydrate pH 4.6	30% v/v (+/-)-2-Methyl-2,4-pentanediol	None
A2	None	None	0.26 M Potassium sodium tartrate tetrahydrate	35% v/v
A3	None	None	0.26 M Ammonium phosphate monobasic	35% v/v
A4	None	0.075 M TRIS hydrochloride pH 8.5	1.5 M Ammonium sulfate	25% v/v
A5	0.2 M Sodium citrate tribasic dihydrate	0.1 M HEPES sodium pH 7.5	30% v/v (+/-)-2-Methyl-2,4-pentanediol	None
A6	0.16 M Magnesium chloride hexahydrate	0.08 M TRIS hydrochloride pH 8.5	24% w/v Polyethylene glycol 4,000	20% v/v
A7	None	0.07 M Sodium cacodylate trihydrate pH 6.5	0.98 M Sodium acetate trihydrate	30% v/v
A8	0.14 M Sodium citrate tribasic dihydrate	0.07 M Sodium cacodylate trihydrate pH 6.5	21% v/v 2-Propanol	30% v/v
A9	0.17 M Ammonium acetate	0.085 M Sodium citrate tribasic dihydrate pH 5.6	25.5% w/v Polyethylene glycol 4,000	15% v/v
A10	0.17 M Ammonium acetate	0.085 M Sodium acetate trihydrate pH 4.6	25.5% w/v Polyethylene glycol 4,000	15% v/v
A11	None	0.07 M Sodium citrate tribasic dihydrate pH 5.6	0.7 M Ammonium phosphate monobasic	30% v/v
A12	0.18 M Magnesium chloride hexahydrate	0.09 M HEPES sodium pH 7.5	27% v/v 2-Propanol	10% v/v
B1	0.2 M Sodium citrate tribasic dihydrate	0.1 M TRIS hydrochloride pH 8.5	30% v/v Polyethylene glycol 400	None
B2	0.19 M Calcium chloride dihydrate	0.095 M HEPES sodium pH 7.5	26.6% v/v Polyethylene glycol 400	5% v/v
B3	0.17 M Ammonium sulfate	0.085 M Sodium cacodylate trihydrate pH 6.5	25.5% w/v Polyethylene glycol 8,000	15% v/v
B4	None	0.075 M HEPES sodium pH 7.5	1.125 M Lithium sulfate monohydrate	25% v/v
B5	0.17 M Lithium sulfate monohydrate	0.085 M TRIS hydrochloride pH 8.5	25.5% w/v Polyethylene glycol 4,000	15% v/v
B6	0.16 M Magnesium acetate tetrahydrate	0.08 M Sodium cacodylate trihydrate pH 6.5	16% w/v Polyethylene glycol 8,000	20% v/v
B7	0.16 M Ammonium acetate	0.08 M TRIS hydrochloride pH 8.5	24% v/v 2-Propanol	20% v/v
B8	0.16 M Ammonium sulfate	0.08 M Sodium acetate trihydrate pH 4.6	20% w/v Polyethylene glycol 4,000	20% v/v
B9	0.2 M Magnesium acetate tetrahydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	30% v/v (+/-)-2-Methyl-2,4-pentanediol	None
B10	0.17 M Sodium acetate trihydrate	0.085 M TRIS hydrochloride pH 8.5	25.5% w/v Polyethylene glycol 4,000	15% v/v
B11	0.2 M Magnesium chloride hexahydrate	0.1 M HEPES sodium pH 7.5	30% v/v Polyethylene glycol 400	None

B12	0.14 M Calcium chloride dihydrate	0.07 M Sodium acetate trihydrate pH 4.6	14% v/v 2-Propanol	30% v/v
C1	None	0.07 M Imidazole pH 6.5	0.7 M Sodium acetate trihydrate	30% v/v
C2	0.2 M Ammonium acetate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	30% v/v (+/-)-2-Methyl-2,4-pentanediol	None
C3	0.14 M Sodium citrate tribasic dihydrate	0.07 M HEPES sodium pH 7.5	14% v/v 2-Propanol	30% v/v
C4	0.17 M Sodium acetate trihydrate	0.085 M Sodium cacodylate trihydrate pH 6.5	25.5% w/v Polyethylene glycol 8,000	15% v/v
C5	None	0.065 M HEPES sodium pH 7.5	0.52 M Potassium sodium tartrate tetrahydrate	35% v/v
C6	0.17 M Ammonium sulfate	None	25.5% w/v Polyethylene glycol 8,000	15% v/v
C7	0.17 M Ammonium sulfate	None	25.5% w/v Polyethylene glycol 4,000	15% v/v
C8	None	None	1.5 M Ammonium sulfate	25% v/v
C9	None	None	3.6 M Sodium formate	10% v/v
C10	None	0.07 M Sodium acetate trihydrate pH 4.6	1.4 M Sodium formate	30% v/v
C11	None	0.075 M HEPES sodium pH 7.5	0.6 M Sodium phosphate monobasic monohydrate, 0.6 M Potassium phosphate monobasic	25% v/v
C12	None	0.065 M TRIS hydrochloride pH 8.5	5.2% w/v Polyethylene glycol 8,000	35% v/v
D1	None	0.07 M Sodium acetate trihydrate pH 4.6	5.6% w/v Polyethylene glycol 4,000	30% v/v
D2	None	0.09 M HEPES sodium pH 7.5	1.26 M Sodium citrate tribasic dihydrate	10% v/v
D3	None	0.085 M HEPES sodium pH 7.5	1.7% v/v Polyethylene glycol 400, 1.7 M Ammonium sulfate	15% v/v
D4	None	0.095 M Sodium citrate tribasic dihydrate pH 5.6	19% v/v 2-Propanol, 19% w/v Polyethylene glycol 4,000	5% v/v
D5	None	0.085 M HEPES sodium pH 7.5	8.5% v/v 2-Propanol, 17% w/v Polyethylene glycol 4,000	15% v/v
D6	0.04 M Potassium phosphate monobasic	None	16% w/v Polyethylene glycol 8,000	20% v/v
D7	None	None	24% w/v Polyethylene glycol 1,500	20% v/v
D8	None	None	0.1 M Magnesium formate dihydrate	50% v/v
D9	0.16 M Zinc acetate dihydrate	0.08 M Sodium cacodylate trihydrate pH 6.5	14.4% w/v Polyethylene glycol 8,000	20% v/v
D10	0.16 M Calcium acetate hydrate	0.08 M Sodium cacodylate trihydrate pH 6.5	14.4% w/v Polyethylene glycol 8,000	20% v/v
D11	None	0.08 M Sodium acetate trihydrate pH 4.6	1.6 M Ammonium sulfate	20% v/v
D12	None	0.08 M TRIS hydrochloride pH 8.5	1.6 M Ammonium phosphate monobasic	20% v/v
E1	1.6 M Sodium chloride	None	8% w/v Polyethylene glycol 6,000	20% v/v
E2	0.3 M Sodium chloride, 0.006 M Magnesium chloride hexahydrate	None	0.006 M Hexadecyltrimethylammonium bromide	40% v/v
E3	None	None	21.25% v/v Ethylene glycol	15% v/v
E4	None	None	26.25% v/v 1,4-Dioxane	25% v/v
E5	1.5 M Ammonium sulfate	None	3.75% v/v 2-Propanol	25% v/v
E6	None	None	0.65 M Imidazole pH 7.0	35% v/v
E7	None	None	8% w/v Polyethylene glycol 1,000, 8% w/v Polyethylene glycol 8,000	20% v/v
E8	1.05 M Sodium chloride	None	7% v/v Ethanol	30% v/v
E9	None	0.075 M Sodium acetate trihydrate pH 4.6	1.5 M Sodium chloride	25% v/v
E10	0.2 M Sodium chloride	0.1 M Sodium acetate trihydrate pH 4.6	30% v/v (+/-)-2-Methyl-2,4-pentanediol	None
E11	0.008 M Cobalt(II) chloride hexahydrate	0.08 M Sodium acetate trihydrate pH 4.6	0.8 M 1,6-Hexanediol	20% v/v
E12	0.095 M Cadmium chloride hydrate	0.095 M Sodium acetate trihydrate pH 4.6	28.5% v/v Polyethylene glycol 400	5% v/v
F1	0.18 M Ammonium sulfate	0.09 M Sodium acetate trihydrate pH 4.6	27% w/v Polyethylene glycol monomethyl ether 2,000	10% v/v
F2	0.15 M Potassium sodium tartrate tetrahydrate	0.075 M Sodium citrate tribasic dihydrate pH 5.6	1.5 M Ammonium sulfate	25% v/v
F3	0.375 M Ammonium sulfate	0.075 M Sodium citrate tribasic dihydrate pH 5.6	0.75 M Lithium sulfate monohydrate	25% v/v

F4	0.3 M Sodium chloride	0.06 M Sodium citrate tribasic dihydrate pH 5.6	1.2% v/v Ethylene imine polymer	40% v/v
F5	None	0.08 M Sodium citrate tribasic dihydrate pH 5.6	28% v/v tert-Butanol	20% v/v
F6	0.007 M Iron(III) chloride hexahydrate	0.07 M Sodium citrate tribasic dihydrate pH 5.6	7% v/v Jeffamine ® M-600 ®	30% v/v
F7	None	0.095 M Sodium citrate tribasic dihydrate pH 5.6	2.375 M 1,6-Hexanediol	5% v/v
F8	None	0.08 M MES monohydrate pH 6.5	1.28 M Magnesium sulfate heptahydrate	20% v/v
F9	0.075 M Sodium phosphate monobasic monohydrate, 0.075 M Potassium phosphate monobasic	0.075 M MES monohydrate pH 6.5	1.5 M Sodium chloride	25% v/v
F10	None	0.065 M MES monohydrate pH 6.5	7.8% w/v Polyethylene glycol 20,000	35% v/v
F11	1.2 M Ammonium sulfate	0.075 M MES monohydrate pH 6.5	7.5% v/v 1,4-Dioxane	25% v/v
F12	0.05 M Cesium chloride	0.1 M MES monohydrate pH 6.5	30% v/v Jeffamine ® M-600 ®	None
G1	0.0075 M Cobalt(II) chloride hexahydrate	0.075 M MES monohydrate pH 6.5	1.35 M Ammonium sulfate	25% v/v
G2	0.18 M Ammonium sulfate	0.09 M MES monohydrate pH 6.5	27% w/v Polyethylene glycol monomethyl ether 5,000	10% v/v
G3	0.009 M Zinc sulfate heptahydrate	0.09 M MES monohydrate pH 6.5	22.5% v/v Polyethylene glycol monomethyl ether 550	10% v/v
G4	None	None	1.6 M Sodium citrate tribasic dihydrate pH 6.5	None
G5	0.5 M Ammonium sulfate	0.1 M HEPES pH 7.5	30% v/v (+/-)-2-Methyl-2,4-pentanediol	None
G6	None	0.08 M HEPES pH 7.5	8% w/v Polyethylene glycol 6,000, 4% v/v (+/-)-2-Methyl-2,4-pentanediol	20% v/v
G7	None	0.085 M HEPES pH 7.5	17% v/v Jeffamine ® M-600 ®	15% v/v
G8	0.075 M Sodium chloride	0.075 M HEPES pH 7.5	1.2 M Ammonium sulfate	25% v/v
G9	None	0.07 M HEPES pH 7.5	1.4 M Ammonium formate	30% v/v
G10	0.0375 M Cadmium sulfate hydrate	0.075 M HEPES pH 7.5	0.75 M Sodium acetate trihydrate	25% v/v
G11	None	0.1 M HEPES pH 7.5	70% v/v (+/-)-2-Methyl-2,4-pentanediol	None
G12	None	0.085 M HEPES pH 7.5	3.655 M Sodium chloride	15% v/v
H1	None	0.075 M HEPES pH 7.5	7.5% w/v Polyethylene glycol 8,000, 6% v/v Ethylene glycol	25% v/v
H2	None	0.075 M HEPES pH 7.5	15% w/v Polyethylene glycol 10,000	25% v/v
H3	0.2 M Magnesium chloride hexahydrate	0.1 M Tris pH 8.5	3.4 M 1,6-Hexanediol	None
H4	None	0.075 M Tris pH 8.5	18.75% v/v tert-Butanol	25% v/v
H5	0.0075 M Nickel(II) chloride hexahydrate	0.075 M Tris pH 8.5	0.75 M Lithium sulfate monohydrate	25% v/v
H6	1.275 M Ammonium sulfate	0.085 M Tris pH 8.5	None	25.2% v/v
H7	0.2 M Ammonium phosphate monobasic	0.1 M Tris pH 8.5	50% v/v (+/-)-2-Methyl-2,4-pentanediol	None
H8	None	0.075 M Tris pH 8.5	15% v/v Ethanol	25% v/v
H9	0.008 M Nickel(II) chloride hexahydrate	0.08 M Tris pH 8.5	16% w/v Polyethylene glycol monomethyl ether 2,000	20% v/v
H10	0.085 M Sodium chloride	0.085 M BICINE pH 9.0	17% v/v Polyethylene glycol monomethyl ether 550	15% v/v
H11	None	0.095 M BICINE pH 9.0	1.9 M Magnesium chloride hexahydrate	5% v/v
H12	None	0.07 M BICINE pH 9.0	1.4% v/v 1,4-Dioxane, 7% w/v Polyethylene glycol 20,000	30% v/v

Clear Strategy Screen I

Well	Salt	Buffer	Precipitant
A1	0.3 M sodium acetate	0.1 M sodium acetate pH 5.5	25% w/v PEG 2K MME
A2	0.2 M lithium sulfate	0.1 M sodium acetate pH 5.5	25% w/v PEG 2K MME
A3	0.2 M magnesium chloride	0.1 M sodium acetate pH 5.5	25% w/v PEG 2K MME
A4	0.2 M potassium bromide	0.1 M sodium acetate pH 5.5	25% w/v PEG 2K MME
A5	0.2 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	25% w/v PEG 2K MME
A6	0.8 M sodium formate	0.1 M sodium acetate pH 5.5	25% w/v PEG 2K MME

A7	0.3 M sodium acetate	0.1 M sodium acetate pH 5.5	15% w/v PEG 4K
A8	0.2 M lithium sulfate	0.1 M sodium acetate pH 5.5	15% w/v PEG 4K
A9	0.2 M magnesium chloride	0.1 M sodium acetate pH 5.5	15% w/v PEG 4K
A10	0.2 M potassium bromide	0.1 M sodium acetate pH 5.5	15% w/v PEG 4K
A11	0.2 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	15% w/v PEG 4K
A12	0.8 M sodium formate	0.1 M sodium acetate pH 5.5	15% w/v PEG 4K
B1	0.3 M sodium acetate	0.1 M sodium acetate pH 5.5	10% w/v PEG 8K + 10% w/v PEG 1K
B2	0.2 M lithium sulfate	0.1 M sodium acetate pH 5.5	10% w/v PEG 8K + 10% w/v PEG 1K
B3	0.2 M magnesium chloride	0.1 M sodium acetate pH 5.5	10% w/v PEG 8K + 10% w/v PEG 1K
B4	0.2 M potassium bromide	0.1 M sodium acetate pH 5.5	10% w/v PEG 8K + 10% w/v PEG 1K
B5	0.2 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	10% w/v PEG 8K + 10% w/v PEG 1K
B6	0.8 M sodium formate	0.1 M sodium acetate pH 5.5	10% w/v PEG 8K + 10% w/v PEG 1K
B7	0.3 M sodium acetate	0.1 M sodium acetate pH 5.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
B8	0.2 M lithium sulfate	0.1 M sodium acetate pH 5.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
B9	0.2 M magnesium chloride	0.1 M sodium acetate pH 5.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
B10	0.2 M potassium bromide	0.1 M sodium acetate pH 5.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
B11	0.2 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
B12	0.8 M sodium formate	0.1 M sodium acetate pH 5.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
C1	0.3 M sodium acetate	0.1 M sodium cacodylate pH 6.5	25% w/v PEG 2K MME
C2	0.2 M lithium sulfate	0.1 M sodium cacodylate pH 6.5	25% w/v PEG 2K MME
C3	0.2 M magnesium chloride	0.1 M sodium cacodylate pH 6.5	25% w/v PEG 2K MME
C4	0.2 M potassium bromide	0.1 M sodium cacodylate pH 6.5	25% w/v PEG 2K MME
C5	0.2 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	25% w/v PEG 2K MME
C6	0.8 M sodium formate	0.1 M sodium cacodylate pH 6.5	25% w/v PEG 2K MME
C7	0.3 M sodium acetate	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 4K
C8	0.2 M lithium sulfate	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 4K
C9	0.2 M magnesium chloride	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 4K
C10	0.2 M potassium bromide	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 4K
C11	0.2 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 4K
C12	0.8 M sodium formate	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 4K
D1	0.3 M sodium acetate	0.1 M sodium cacodylate pH 6.5	10% w/v PEG 8K + 10% w/v PEG 1K
D2	0.2 M lithium sulfate	0.1 M sodium cacodylate pH 6.5	10% w/v PEG 8K + 10% w/v PEG 1K
D3	0.2 M magnesium chloride	0.1 M sodium cacodylate pH 6.5	10% w/v PEG 8K + 10% w/v PEG 1K
D4	0.2 M potassium bromide	0.1 M sodium cacodylate pH 6.5	10% w/v PEG 8K + 10% w/v PEG 1K
D5	0.2 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	10% w/v PEG 8K + 10% w/v PEG 1K
D6	0.8 M sodium formate	0.1 M sodium cacodylate pH 6.5	10% w/v PEG 8K + 10% w/v PEG 1K
D7	0.3 M sodium acetate	0.1 M sodium cacodylate pH 6.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
D8	0.2 M lithium sulfate	0.1 M sodium cacodylate pH 6.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
D9	0.2 M magnesium chloride	0.1 M sodium cacodylate pH 6.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
D10	0.2 M potassium bromide	0.1 M sodium cacodylate pH 6.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
D11	0.2 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
D12	0.8 M sodium formate	0.1 M sodium cacodylate pH 6.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
E1	0.3 M sodium acetate	0.1 M Tris pH 7.5	25% w/v PEG 2K MME
E2	0.2 M lithium sulfate	0.1 M Tris pH 7.5	25% w/v PEG 2K MME
E3	0.2 M magnesium chloride	0.1 M Tris pH 7.5	25% w/v PEG 2K MME
E4	0.2 M potassium bromide	0.1 M Tris pH 7.5	25% w/v PEG 2K MME
E5	0.2 M potassium thiocyanate	0.1 M Tris pH 7.5	25% w/v PEG 2K MME
E6	0.8 M sodium formate	0.1 M Tris pH 7.5	25% w/v PEG 2K MME
E7	0.3 M sodium acetate	0.1 M Tris pH 7.5	15% w/v PEG 4K
E8	0.2 M lithium sulfate	0.1 M Tris pH 7.5	15% w/v PEG 4K
E9	0.2 M magnesium chloride	0.1 M Tris pH 7.5	15% w/v PEG 4K
E10	0.2 M potassium bromide	0.1 M Tris pH 7.5	15% w/v PEG 4K

E11	0.2 M potassium thiocyanate	0.1 M Tris pH 7.5	15% w/v PEG 4K
E12	0.8 M sodium formate	0.1 M Tris pH 7.5	15% w/v PEG 4K
F1	0.3 M sodium acetate	0.1 M Tris pH 7.5	10% w/v PEG 8K + 10% w/v PEG 1K
F2	0.2 M lithium sulfate	0.1 M Tris pH 7.5	10% w/v PEG 8K + 10% w/v PEG 1K
F3	0.2 M magnesium chloride	0.1 M Tris pH 7.5	10% w/v PEG 8K + 10% w/v PEG 1K
F4	0.2 M potassium bromide	0.1 M Tris pH 7.5	10% w/v PEG 8K + 10% w/v PEG 1K
F5	0.2 M potassium thiocyanate	0.1 M Tris pH 7.5	10% w/v PEG 8K + 10% w/v PEG 1K
F6	0.8 M sodium formate	0.1 M Tris pH 7.5	10% w/v PEG 8K + 10% w/v PEG 1K
F7	0.3 M sodium acetate	0.1 M Tris pH 7.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
F8	0.2 M lithium sulfate	0.1 M Tris pH 7.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
F9	0.2 M magnesium chloride	0.1 M Tris pH 7.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
F10	0.2 M potassium bromide	0.1 M Tris pH 7.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
F11	0.2 M potassium thiocyanate	0.1 M Tris pH 7.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
F12	0.8 M sodium formate	0.1 M Tris pH 7.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
G1	0.3 M sodium acetate	0.1 M Tris pH 8.5	25% w/v PEG 2K MME
G2	0.2 M lithium sulfate	0.1 M Tris pH 8.5	25% w/v PEG 2K MME
G3	0.2 M magnesium chloride	0.1 M Tris pH 8.5	25% w/v PEG 2K MME
G4	0.2 M potassium bromide	0.1 M Tris pH 8.5	25% w/v PEG 2K MME
G5	0.2 M potassium thiocyanate	0.1 M Tris pH 8.5	25% w/v PEG 2K MME
G6	0.8 M sodium formate	0.1 M Tris pH 8.5	25% w/v PEG 2K MME
G7	0.3 M sodium acetate	0.1 M Tris pH 8.5	15% w/v PEG 4K
G8	0.2 M lithium sulfate	0.1 M Tris pH 8.5	15% w/v PEG 4K
G9	0.2 M magnesium chloride	0.1 M Tris pH 8.5	15% w/v PEG 4K
G10	0.2 M potassium bromide	0.1 M Tris pH 8.5	15% w/v PEG 4K
G11	0.2 M potassium thiocyanate	0.1 M Tris pH 8.5	15% w/v PEG 4K
G12	0.8 M sodium formate	0.1 M Tris pH 8.5	15% w/v PEG 4K
H1	0.3 M sodium acetate	0.1 M Tris pH 8.5	10% w/v PEG 8K + 10% w/v PEG 1K
H2	0.2 M lithium sulfate	0.1 M Tris pH 8.5	10% w/v PEG 8K + 10% w/v PEG 1K
H3	0.2 M magnesium chloride	0.1 M Tris pH 8.5	10% w/v PEG 8K + 10% w/v PEG 1K
H4	0.2 M potassium bromide	0.1 M Tris pH 8.5	10% w/v PEG 8K + 10% w/v PEG 1K
H5	0.2 M potassium thiocyanate	0.1 M Tris pH 8.5	10% w/v PEG 8K + 10% w/v PEG 1K
H6	0.8 M sodium formate	0.1 M Tris pH 8.5	10% w/v PEG 8K + 10% w/v PEG 1K
H7	0.3 M sodium acetate	0.1 M Tris pH 8.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
H8	0.2 M lithium sulfate	0.1 M Tris pH 8.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
H9	0.2 M magnesium chloride	0.1 M Tris pH 8.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
H10	0.2 M potassium bromide	0.1 M Tris pH 8.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
H11	0.2 M potassium thiocyanate	0.1 M Tris pH 8.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
H12	0.8 M sodium formate	0.1 M Tris pH 8.5	8% w/v PEG 20K + 8% v/v PEG 550 MME

Clear Strategy Screen II

Well	Salt	Buffer	Precipitant
A1	1.5 M ammonium sulfate	0.1 M sodium acetate pH 5.5	None
A2	0.8 M lithium sulfate	0.1 M sodium acetate pH 5.5	None
A3	2.0 M sodium formate	0.1 M sodium acetate pH 5.5	None
A4	0.5 M potassium dihyd. phosphate	0.1 M sodium acetate pH 5.5	None
A5	0.2 M calcium acetate	0.1 M sodium acetate pH 5.5	25% w/v PEG 2K MME
A6	0.2 M calcium acetate	0.1 M sodium acetate pH 5.5	15% w/v PEG 4K
A7	2.7 M ammonium sulfate	0.1 M sodium acetate pH 5.5	None
A8	1.8 M lithium sulfate	0.1 M sodium acetate pH 5.5	None
A9	4.0 M sodium formate	0.1 M sodium acetate pH 5.5	None
A10	1.0 M potassium dihyd. phosphate	0.1 M sodium acetate pH 5.5	None
A11	0.2 M calcium acetate	0.1 M sodium acetate pH 5.5	10% w/v PEG 8K + 10% w/v PEG 1K

A12	0.2 M calcium acetate	0.1 M sodium acetate pH 5.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
B1	None	0.1 M sodium acetate pH 5.5	40% v/v MPD
B2	None	0.1 M sodium acetate pH 5.5	40% v/v 1,4-Butanediol
B3	0.005 M cadmium chloride	0.1 M sodium acetate pH 5.5	20% w/v PEG 4K
B4	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	20% v/v PEG 550 MME
B5	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	20% v/v PEG 600
B6	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	20% w/v PEG 1.5K
B7	None	0.1 M sodium acetate pH 5.5	35% v/v 2-Propanol
B8	None	0.1 M sodium acetate pH 5.5	30% v/v Jeffamine M-600
B9	0.005 M nickel chloride	0.1 M sodium acetate pH 5.5	20% w/v PEG 4K
B10	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	18% w/v PEG 3350
B11	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	18% w/v PEG 5K MME
B12	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	15% w/v PEG 6K
C1	1.5 M ammonium sulfate	0.1 M sodium cacodylate pH 6.5	None
C2	0.8 M lithium sulfate	0.1 M sodium cacodylate pH 6.5	None
C3	2.0 M sodium formate	0.1 M sodium cacodylate pH 6.5	None
C4	0.5 M potassium dihyd. phosphate	0.1 M sodium cacodylate pH 6.5	None
C5	0.2 M calcium acetate	0.1 M sodium cacodylate pH 6.6	25% w/v PEG 2K MME
C6	0.2 M calcium acetate	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 4K
C7	2.7 M ammonium sulfate	0.1 M sodium cacodylate pH 6.5	None
C8	1.8 M lithium sulfate	0.1 M sodium cacodylate pH 6.5	None
C9	4.0 M sodium formate	0.1 M sodium cacodylate pH 6.5	None
C10	1.0 M potassium dihyd. phosphate	0.1 M sodium cacodylate pH 6.5	None
C11	0.2 M calcium acetate	0.1 M sodium cacodylate pH 6.5	10% w/v PEG 8K + 10% w/v PEG 1K
C12	0.2 M calcium acetate	0.1 M sodium cacodylate pH 6.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
D1	None	0.1 M sodium cacodylate pH 6.5	40% v/v MPD
D2	None	0.1 M sodium cacodylate pH 6.5	40% v/v 1,4-Butanediol
D3	0.005 M cadmium chloride	0.1 M sodium cacodylate pH 6.5	20% w/v PEG 4K
D4	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	20% v/v PEG 550 MME
D5	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	20% v/v PEG 600
D6	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	20% w/v PEG 1.5K
D7	None	0.1 M sodium cacodylate pH 6.5	35% v/v 2-Propanol
D8	None	0.1 M sodium cacodylate pH 6.5	30% v/v Jeffamine M-600
D9	0.005 M nickel chloride	0.1 M sodium cacodylate pH 6.5	20% w/v PEG 4K
D10	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	18% w/v PEG 3350
D11	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	18% w/v PEG 5K MME
D12	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 6K
E1	1.5 M ammonium sulfate	0.1M Tris pH 7.5	None
E2	0.8 M lithium sulfate	0.1M Tris pH 7.5	None
E3	2.0 M sodium formate	0.1M Tris pH 7.5	None
E4	0.5 M potassium dihyd. phosphate	0.1M Tris pH 7.5	None
E5	0.2 M calcium acetate	0.1M Tris pH 7.5	25% w/v PEG 2K MME
E6	0.2 M calcium acetate	0.1M Tris pH 7.5	15% w/v PEG 4K
E7	2.7 M ammonium sulfate	0.1M Tris pH 7.5	None
E8	1.8 M lithium sulfate	0.1M Tris pH 7.5	None
E9	4.0 M sodium formate	0.1M Tris pH 7.5	None
E10	1.0 M potassium dihyd. phosphate	0.1M Tris pH 7.5	None
E11	0.2 M calcium acetate	0.1M Tris pH 7.5	10% w/v PEG 8K + 10% w/v PEG 1K
E12	0.2 M calcium acetate	0.1M Tris pH 7.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
F1	None	0.1M Tris pH 7.5	40% v/v MPD
F2	None	0.1M Tris pH 7.5	40% v/v 1,4-Butanediol
F3	0.005 M cadmium chloride	0.1M Tris pH 7.5	20% w/v PEG 4K

F4	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	20% v/v PEG 550 MME
F5	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	20% w/v PEG 600
F6	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	20% w/v PEG 1.5K
F7	None	0.1M Tris pH 7.5	35% v/v 2-Propanol
F8	None	0.1M Tris pH 7.5	30% v/v Jeffamine M-600
F9	0.005 M nickel chloride	0.1M Tris pH 7.5	20% w/v PEG 4K
F10	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	18% w/v PEG 3350
F11	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	18% w/v PEG 5K MME
F12	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	15% w/v PEG 6K
G1	1.5 M ammonium sulfate	0.1M Tris pH 8.5	None
G2	0.8 M lithium sulfate	0.1M Tris pH 8.5	None
G3	2.0 M sodium formate	0.1M Tris pH 8.5	None
G4	0.5 M potassium dihyd. phosphate	0.1M Tris pH 8.5	None
G5	0.2 M calcium acetate	0.1M Tris pH 8.5	25% w/v PEG 2K MME
G6	0.2 M calcium acetate	0.1M Tris pH 8.5	15% w/v PEG 4K
G7	2.7 M ammonium sulfate	0.1M Tris pH 8.5	None
G8	1.8 M lithium sulfate	0.1M Tris pH 8.5	None
G9	4.0 M sodium formate	0.1M Tris pH 8.5	None
G10	1.0 M potassium dihyd. phosphate	0.1M Tris pH 8.5	None
G11	0.2 M calcium acetate	0.1M Tris pH 8.5	10% w/v PEG 8K + 10% w/v PEG 1K
G12	0.2 M calcium acetate	0.1M Tris pH 8.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
H1	None	0.1M Tris pH 8.5	40% v/v MPD
H2	None	0.1M Tris pH 8.5	40% v/v 1,4-Butanediol
H3	0.005 M cadmium chloride	0.1M Tris pH 8.5	20% w/v PEG 4K
H4	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	20% v/v PEG 550 MME
H5	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	20% v/v PEG 600
H6	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	20% w/v PEG 1.5K
H7	None	0.1M Tris pH 8.5	35% v/v 2-Propanol
H8	None	0.1M Tris pH 8.5	30% v/v Jeffamine M-600
H9	0.005 M nickel chloride	0.1M Tris pH 8.5	20% w/v PEG 4K
H10	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	18% w/v PEG 3350
H11	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	18% w/v PEG 5K MME
H12	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	15% w/v PEG 6K

JCSG Core Suite I

Well	Salt	Buffer	Precipitant	Final pH
A1		0.1 M CHES pH 9.5	20 %(w/v) PEG 8000	
A2		0.1 M Bicine pH 8.5	20 %(w/v) PEG 6000	9,0
A3	0.05 M Lithium sulfate, 0.05 M Sodium sulfate	0.05 M Tris-HCl pH 8.5	30 %(w/v) PEG 400	
A4	0.2 M Ammonium dihydrogen phosphate	0.1 M Tris pH 8.5	50 %(v/v) MPD	
A5	0.2 M Magnesium chloride	0.1 M Tris pH 8.5	3.4 M 1,6 Hexanediol	
A6	0.05 M Magnesium chloride	0.1M Tris pH 8.5	40%(v/v) Ethanol	
A7		0.2 M tri-Potassium citrate	20%(w/v) PEG 3350	
A8		0.2 M tri-Sodium citrate	20%(w/v) PEG 3350	
A9		0.2 M tri-Lithium citrate	20%(w/v) PEG 3350	
A10	0.2 M Calcium acetate	0.1 M Imidazole pH 8.0	20 %PEG 1000	
A11		0.2 M Potassium acetate	20%(w/v) PEG 3350	
A12		0.2 M Magnesium acetate	20%(w/v) PEG 3350	
B1	0.2 M Sodium chloride	0.1 M HEPES pH 7.5	20 %(w/v) PEG 3000	
B2		0.1 M HEPES pH 7.5	20 %(w/v) PEG 8000	
B3		0.1 M HEPES pH 7.5	10 %(w/v) PEG 8000	
B4	0.19 M Calcium chloride	0.095 M HEPES pH 7.5	26.6%(v/v) PEG 400, 5 %(v/v)	

			Glycerol	
B5		0.1 M HEPES pH 7.5	20 %(w/v) PEG 4000,10 %(v/v) Isopropanol	
B6	0.8 M di-Sodium hydrogen phosphate,0.8 M di-Potassium hydrogen phosphate	0.1 M HEPES pH 7.5		
B7	0.2 M di-Sodium tartrate		20%(w/v) PEG 3350	
B8	0.2 M Calcium acetate hydrate		20%(w/v) PEG 3350	
B9	0.2 M Potassium formate		20%(w/v) PEG 3350	
B10	0.2 M Potassium Sodium tartrate		20%(w/v) PEG 3350	
B11	0.2 M Sodium formate		20%(w/v) PEG 3350	
B12	0.2 M Potassium fluoride		20%(w/v) PEG 3350	
C1	0.2 M Ammonium acetate		20%(w/v) PEG 3350	
C2	0.2 M Lithium nitrate		20%(w/v) PEG 3350	
C3		0.1M Sodium cacodylate pH 6.5	5%(w/v) PEG 8000,40%(v/v) MPD	
C4	0.2 M Magnesium chloride	0.1 M Tris pH 7.0	10 %(w/v) PEG 8000	
C5	0.2 M Calcium acetate	0.1 M Tris pH 7.0	20 %(w/v) PEG 3000	
C6	0.2 M Magnesium chloride	0.1 M Tris pH 7.0	2.5 M Sodium chloride	
C7		0.1 M Tris pH 7.0	20 %(w/v) PEG 2000 MME	
C8	0.2 M Sodium acetate		20%(w/v) PEG 3350	
C9	0.2 M Potassium thiocyanate		20%(w/v) PEG 3350	
C10		0.1 M HEPES pH 6.5	20 %(w/v) PEG 6000	7,0
C11	0.2 M Potassium nitrate		20%(w/v) PEG 3350	
C12	0.2 M Sodium thiocyanate		20%(w/v) PEG 3350	
D1	0.2 M Sodium iodide		20%(w/v) PEG 3350	
D2	0.2 M Potassium chloride		20%(w/v) PEG 3350	
D3	0.2 M Sodium chloride		20%(w/v) PEG 3350	
D4	0.2 M Potassium iodide		20%(w/v) PEG 3350	
D5	0.2 M Lithium chloride		20%(w/v) PEG 3350	
D6	0.2 M Magnesium chloride	0.1M Sodium cacodylate pH 6.5	50%(v/v) PEG 200	
D7	0.2 M di-Ammonium tartrate		20%(w/v) PEG 3350	
D8	0.2 M Sodium sulfate		20%(w/v) PEG 3350	
D9	0.2 M Ammonium formate		20%(w/v) PEG 3350	
D10		0.1 M HEPES pH 7.5	10 %(w/v) PEG 6000, 5 %(v/v) MPD	
D11		1.6 M Sodium citrate pH 6.5		
D12	0.2 M Magnesium acetate	0.1 M Sodium cacodylate pH 6.5	20 %(w/v) PEG 8000	
E1	0.2 M Ammonium nitrate		20%(w/v) PEG 3350	
E2	0.2 M Ammonium chloride		20%(w/v) PEG 3350	
E3	0.2 M Sodium chloride	0.1 M Na/K phosphate pH 6.2	10 %(w/v) PEG 8000	
E4	0.2 M Ammonium iodide		20%(w/v) PEG 3350	
E5	0.2 M Ammonium fluoride		20%(w/v) PEG 3350	
E6		0.1M MES pH 6.0	5%(w/v) PEG 3000,30%(v/v) PEG 200	
E7	0.2 M Calcium acetate	0.1 M MES pH 6.0	20 %(w/v) PEG 8000	
E8	0.2 M Lithium sulfate	0.1 M MES pH 6.0	35 %(v/v) MPD	
E9	0.2 M Ammonium sulfate		20%(w/v) PEG 3350	
E10		0.1 M MES pH 5.0	40 %(v/v) MPD	6,0
E11		0.1 M MES pH 5.0	20 %(v/v) MPD	6,0
E12		0.1 M MES pH 5.0	20 %(w/v) PEG 6000	6,0
F1		0.1 M MES pH 5.0	10 %(w/v) PEG 6000	6,0
F2	0.2 M Magnesium sulfate		20%(w/v) PEG 3350	
F3	0.2 M Magnesium formate		20%(w/v) PEG 3350	
F4	0.2 M Magnesium nitrate		20%(w/v) PEG 3350	
F5	0.2 M Magnesium chloride		20%(w/v) PEG 3350	

F6		0.095 M Sodium citrate pH 5.6	19 %(v/v) Isopropanol,19 %(w/v) PEG 4000, 5 %(v/v) Glycerol	
F7		0.1 M Sodium citrate pH 5.6	20 %(v/v) Isopropanol,20 %(w/v) PEG 4000	
F8		0.1 M Sodium citrate pH 5.5	20 %(w/v) PEG 3000	
F9	0.2 M Sodium chloride	0.1M Phosphate-citrate pH 4.2	50%(v/v) PEG 200	
F10		0.1M Phosphate-citrate pH 4.2	5%(w/v) PEG 1000,40% Ethanol	
F11	0.2 M Lithium sulfate	0.1M Sodium acetate pH 4.5	50%(v/v) PEG 400	
F12		0.1M Phosphate-citrate pH 4.2	40%(v/v) MPD	
G1		0.18 M tri-Ammonium citrate	20%(w/v) PEG 3350	
G2		0.1 M Sodium acetate pH 5.0	20 %(v/v) MPD	
G3	1.0 M Lithium chloride	0.1 M Citric Acid pH 5.0	10 %(w/v) PEG 6000	5,0
G4		0.1 M Citric Acid pH 4.0	20 %(w/v) PEG 6000	5,0
G5		0.1 M Citric Acid	10 %(w/v) PEG 6000	5,0
G6		0.1 M Citric Acid pH 4.0	5 %(w/v) PEG 6000	5,0
G7	0.2 M Potassium dihydrogen phosphate		20%(w/v) PEG 3350	
G8	0.2 M Ammonium dihydrogen phosphate		20%(w/v) PEG 3350	
G9	0.2 M Ammonium sulfate	0.1 M Sodium acetate pH 4.6	30 %(w/v) PEG 2000 MME	
G10		0.1 M Sodium acetate pH 4.6	8 %(w/v) PEG 4000	
G11	0.2 M Ammonium sulfate	0.1 M Sodium acetate pH 4.6	25 %(w/v) PEG 4000	
G12	0.02 M Calcium chloride	0.1 M Sodium acetate pH 4.6	30 %(v/v) MPD	
H1		0.1 M Sodium acetate pH 4.5	35 %(v/v) MPD	
H2		0.1 M Sodium acetate pH 4.5	20 %(w/v) PEG 3000	
H3	0.2 M Sodium dihydrogen phosphate		20%(w/v) PEG 3350	
H4	0.05 M Potassium dihydrogen phosphate		20 %(w/v) PEG 8000	
H5	0.2 M Sodium chloride	0.1 M Phosphate-citrate pH 4.2	10 %(w/v) PEG 3000	
H6		0.1 M Phosphate/citrate pH 4.2	2.0 M Ammonium sulfate	
H7	0.2 M Lithium sulfate	0.1 M Phosphate-citrate pH 4.2	20 %(w/v) PEG 1000	
H8		0.1 M Citric Acid pH 2.5	20 %(v/v) MPD	4,0
H9		0.1 M Citric Acid pH 3.5	0.8 M Ammonium sulfate	4,0
H10	1.0 M Lithium chloride	0.1 M Citric Acid pH 4.0	20 %(w/v) PEG 6000	4,0
H11	1.0 M Lithium chloride	0.1 M Citric Acid pH 4.0	10 %(w/v) PEG 6000	4,0
H12		0.1 M Citric Acid pH 4.0	5 %(w/v) PEG 6000	4,0

JCSG Core Suite II

Well	Salt	Buffer	Precipitant	final pH
A1	0.2 M Sodium chloride	0.1 M CAPS pH 10.5	20%(w/v) PEG 8000	
A2	0.2 M Sodium chloride	0.1 M CHES pH 9.5	1.26 M Ammonium sulfate	
A3	1.0 M Sodium citrate	0.1 M CHES pH 9.5		
A4	0.2 M Sodium chloride	0.1 M CHES pH 9.5	10%(w/v) PEG 8000	
A5		0.1 M Bicine pH 9.0	10%(w/v) PEG 20000, 2%(v/v) 1,4-Dioxane	
A6	0.1 M Sodium chloride	0.1 M Bicine pH 9.0	20%(w/v) PEG 550 MME	
A7	1.0 M Lithium chloride	0.1 M Bicine pH 9.0	10%(w/v) PEG 6000	9,0
A8		0.1M Tris pH 8.5	5%(w/v) PEG 8000,20%(v/v) PEG 300, 10%(v/v) Glycerol	
A9	0.01 M Nickel chloride	0.1 M Tris pH 8.5	20%(w/v) PEG 2000 MME	
A10		0.1 M Tris pH 8.5	20%(v/v) Ethanol	
A11		0.1 M Tris-HCl pH 8.5	2.0 M Ammonium dihydrogen phosphate	
A12		0.1 M Tris-HCl pH 8.5	8%(w/v) PEG 8000	
B1		0.1 M Tris-HCl pH 8.5	2.0 M Ammonium sulfate	
B2	0.2 M Lithium sulfate	0.1M Tris pH 8.5	40%(v/v) PEG 400	
B3	0.2 M Calcium acetate	0.1 M Imidazole pH 8.0	10%(w/v) PEG 8000	

B4	0.2 M Magnesium chloride	0.1 M Imidazole pH 8.0	35%(v/v) MPD	
B5	1.0 M Lithium chloride	0.1 M Tris pH 8.5	20%(w/v) PEG 6000	8,0
B6		0.1 M Tris pH 8.5	20%(w/v) PEG 6000	8,0
B7	0.2 M Lithium Acetate		20%(w/v) PEG 3350	
B8	0.2 M Magnesium chloride	0.1M Imidazole pH 8.0	40%(v/v) MPD	
B9	0.2 M Magnesium chloride	0.1 M HEPES pH 7.5	15%(v/v) Ethanol	
B10		0.1 M HEPES pH 7.5	70%(v/v) MPD	
B11		0.085 M Sodium HEPES pH 7.5	17%(w/v) PEG 4000, 15%(v/v) Glycerol,8.5%(v/v) Isopropanol	
B12	0.6 M sodium dihydrogen phosphate/0.6 M potassium dihydrogen phosphate	0.075 M Sodium HEPES pH 7.5	25%(v/v) Glycerol	
C1	0.18 M Magnesium chloride	0.09 M Sodium HEPES pH 7.5	27%(v/v) PEG 400,10%(v/v) Glycerol	
C2		0.1 M Sodium HEPES pH 7.5	2%(v/v) PEG 400,2.0 M Ammonium sulfate	
C3	0.2 M Magnesium chloride	0.1 M Sodium HEPES pH 7.5	30%(v/v) PEG 400	
C4	0.2 M Sodium chloride	0.1M Na/K phosphate pH 6.2	50%(v/v) PEG 200	
C5	0.2 M Sodium fluoride		20%(w/v) PEG 3350	
C6	0.2 M Lithium sulfate	0.1 M Tris pH 7.0	2.0 M Ammonium sulfate	
C7	0.2 M Calcium acetate	0.1M Sodium cacodylate pH 6.5	40%(v/v) PEG 300	
C8		0.1 M Tris pH 7.0	20%(w/v) PEG 1000	
C9	1.0 M Lithium chloride	0.1 M HEPES pH 7.0	10%(w/v) PEG 6000	7,0
C10		0.1 M HEPES pH 6.5	10%(w/v) PEG 6000	7,0
C11	0.2 M Sodium chloride	0.1M Na/K phosphate pH 6.2	40%(v/v) PEG 400	
C12		0.1M Sodium citrate pH 5.5	50%(v/v) PEG 200	
D1		0.1M Na/K phosphate pH 6.2	25%(v/v) 1,2-Propanediol, 10%(v/v) Glycerol	
D2	0.2 M Sodium nitrate		20%(w/v) PEG 3350	
D3	0.05 M Lithium sulfate	0.1M Tris pH 7.0	50%(v/v) PEG 200	
D4	0.2 M Potassium sulfate		20%(w/v) PEG 3350	
D5	0.2 M Magnesium formate			
D6		0.1MSodium citrate pH 5.5	40%(v/v) PEG 600	
D7	0.2 M Magnesium chloride	0.1 M Sodium cacodylate pH 6.5	20%(w/v) PEG 1000	
D8	0.2 M Magnesium chloride	0.1 M Sodium cacodylate pH 6.5	10%(w/v) PEG 3000	
D9	0.2 M Lithium sulfate	0.1 M Sodium cacodylate pH 6.5	30%(v/v) PEG 400	
D10	0.2 M Sodium chloride	0.1 M Sodium cacodylate pH 6.5	2.0 M Ammonium sulfate	
D11		0.1 M MES pH 6.5	12%(w/v) PEG 20000	
D12	0.2 M Lithium sulfate		20%(w/v) PEG 3350	
E1	0.2 M Sodium chloride	0.1 M Na/K phosphate pH 6.2	20%(w/v) PEG 1000	
E2		0.1 M MES pH 5.0	10%(v/v) MPD	6,0
E3	1.0 M Lithium chloride	0.1 M MES pH 6.0	20%(w/v) PEG 6000	6,0
E4	1.0 M Lithium chloride	0.1 M MES pH 6.0	10%(w/v) PEG 6000	6,0
E5		0.1 M MES pH 5.0	5%(w/v) PEG 6000	6,0
E6	0.2 M Zinc acetate	0.1M Imidazole pH 8.0	25%(v/v) 1,2-Propanediol, 10%(v/v) Glycerol	
E7	0.2 M Zinc acetate	0.1M Imidazole pH 8.0	40%(v/v) PEG 600	
E8	0.5 M Ammonium sulfate	0.1M Tris pH 7.0	30%(v/v) PEG 600, 10%(v/v) Glycerol	
E9	1.0 M Lithium sulfate	0.1 M Sodium citrate pH 5.6	0.5 M Ammonium sulfate	
E10	0.2 M Ammonium acetate	0.1 M Sodium citrate pH 5.6	30%(w/v) PEG 4000	
E11			24%(w/v) PEG 1500, 20%(v/v) Glycerol	
E12	0.2 M Sodium chloride	0.1M Sodium acetate pH 4.5	40%(v/v) PEG 300	
F1		0.1M Sodium acetate pH 4.5	35%(v/v) MPD, 10%(v/v) Glycerol	
F2		0.1M Phosphate-citrate pH 4.2	40%(v/v) PEG 300	
F3		0.1M Sodium acetate pH 4.5	5%(w/v) PEG 1000,50%(v/v) Ethylene glycol	

F4	0.1 M Sodium chloride	0.1M Sodium acetate pH 4.5	30%(v/v) PEG 200	
F5		0.1M Sodium acetate pH 4.5	40%(v/v) 1,2-Propanediol	
F6		0.1M Sodium acetate pH 4.5	40%(v/v) Ethylene glycol	
F7		0.1 M Sodium acetate pH 5.0	10%(v/v) MPD	5,0
F8		0.1 M Citric acid pH 4.0	2.4 M Ammonium sulfate	5,0
F9		0.1 M Citric acid pH 4.0	1.6 M Ammonium sulfate	5,0
F10		0.1 M Citric acid pH 4.0	0.8 M Ammonium sulfate	5,0
F11	1.0 M Lithium chloride	0.1 M Citric acid pH 5.0	20%(w/v) PEG 6000	5,0
F12		0.1M Phosphate-citrate pH 4.2	5%(w/v) PEG 3000,25%(v/v) 1,2-Propanediol, 10%(v/v) Glycerol	
G1			2.0 M Ammonium sulfate,5%(v/v) Isopropanol	
G2			2.0 M Ammonium sulfate	
G3	0.2 M Magnesium chloride	0.1M MES pH 5.5	40%(v/v) PEG 400	
G4	0.01 M Cobalt chloride	0.1 M Sodium acetate pH 4.6	1.0 M Hexanediol	
G5		0.08 M Sodium acetate pH 4.6	1.6 M Ammonium sulfate, 20%(v/v) Glycerol	
G6		0.07 M Sodium acetate pH 4.6	5.6%(w/v) PEG 4000, 30%(v/v) Glycerol	
G7	0.14 M Calcium chloride	0.07 M Sodium acetate pH 4.6	30%(v/v) Glycerol, 14%(v/v) Isopropanol	
G8	0.16 M Ammonium sulfate	0.08 M Sodium acetate pH 4.6	20%(w/v) PEG 4000, 20%(v/v) Glycerol	
G9	0.018 M Calcium chloride	0.09 M Sodium acetate pH 4.6	27%(v/v) MPD, 10%(v/v) Glycerol	
G10		0.1 M Sodium acetate pH 4.6	2.0 M Ammonium sulfate	
G11	0.2 M Zinc acetate	0.1 M Sodium acetate pH 4.5	10%(w/v) PEG 3000	
G12	0.2 M Ammonium sulfate	0.1M Phosphate-citrate pH 4.2	20%(v/v) PEG 300, 10% Glycerol	
H1	0.2 M Calcium acetate	0.1 M Sodium acetate pH 4.5	30%(v/v) PEG 400	
H2	0.2 M Lithium sulfate	0.1 M Sodium acetate pH 4.5	30%(w/v) PEG 8000	
H3			25%(v/v) Ethylene glycol	
H4	0.2 M Lithium sulfate	0.1 M Phosphate-citrate pH 4.2	10%(v/v) Isopropanol	
H5	0.2 M Sodium chloride	0.1 M Phosphate-citrate pH 4.2	20%(w/v) PEG 8000	
H6			10%(w/v) PEG 1000, 10%(w/v) PEG 8000	
H7	0.17 M Ammonium sulfate		25.5%(w/v) PEG 4000, 15%(v/v) Glycerol	
H8			30%(w/v) PEG 1500	
H9	0.4 M Ammonium dihydrogen phosphate			
H10			35%(v/v) 1,4-Dioxane	
H11		0.1 M Citric acid pH 2.5	10%(v/v) MPD	4,0
H12		0.1 M Citric acid pH 2.5	20%(w/v) PEG 6000	4,0

JCSG Core Suite III

Well	Salt	Buffer	Precipitant	Final pH
A1		0.1 M CAPS pH 10.5	30%(v/v) PEG 400	
A2		0.1M CHES pH 9.5	40% (v/v) PEG 600	
A3		0.1M CHES pH 9.5	50% (v/v) PEG 200	
A4		0.1M CHES pH 9.5	30%(w/v) PEG 3000	
A5	0.2 M Sodium chloride	0.1M CHES pH 9.5	50%(v/v) PEG 400	
A6	0.2 M di-Potassium hydrogen phosphate		20%(w/v) PEG 3350	
A7	0.2 M di-Sodium hydrogen phosphate		20%(w/v) PEG 3350	
A8		0.1 M Bicine pH 8.5	40%(v/v) MPD	9,0
A9		0.1 M Bicine pH 8.5	5%(w/v) PEG 6000	9,0
A10	0.2 M Ammonium sulfate	0.1M CAPS pH 10.5	30% (v/v) PEG 200	
A11		0.1 M Tris pH 8.5	20%(w/v) PEG 1000	
A12		0.1 M Tris pH 8.5	1.0 M di-Ammonium hydrogen phosphate	

B1	0.2 M Magnesium chloride	0.1 M Tris pH 8.5	20%(w/v) PEG 8000	
B2	0.2 M Lithium sulfate	0.1 M Tris pH 8.5	1.26 M Ammonium sulfate	
B3	0.01 M Nickel chloride	0.1 M Tris pH 8.5	1.0 M Lithium sulfate	
B4	1.6 M Ammonium dihydrogen phosphate	0.08 M Tris-HCl pH 8.5	20%(v/v) Glycerol	
B5	0.2 M Sodium acetate	0.1 M Tris·HCl pH 8.5	30%(w/v) PEG 4000	
B6	1.0 M Sodium citrate	0.1 M Imidazole pH 8.0		
B7	0.2 M Magnesium chloride	0.1 M Imidazole pH 8.0	15%(v/v) Ethanol	
B8	0.2 M Lithium sulfate	0.1 M Imidazole pH 8.0	10%(w/v) PEG 3000	
B9		0.1 M Tris pH 8.5	40%(v/v) MPD	8,0
B10		0.1 M Tris pH 8.5	2.4 M Ammonium sulfate	8,0
B11	0.2 M di-Ammonium hydrogen phosphate		20%(w/v) PEG 3350	
B12	0.2 M Sodium chloride	0.1 M HEPES pH 7.5	30%(v/v) PEG 400	
C1	0.05 M Calcium acetate	0.1M Imidazole pH 8.0	35%(v/v) 2-Ethoxyethanol	
C2	0.2 M tri-Sodium citrate	0.1 M HEPES pH 7.5	10%(v/v) Isopropanol	
C3	0.1 M Sodium chloride	0.1 M HEPES pH 7.5	1.6 M Ammonium sulfate	
C4	0.18 M Magnesium chloride	0.09 M Sodium HEPES pH 7.5	10%(v/v) Glycerol,27%(v/v) Isopropanol	
C5	1.4 M tri-Sodium citrate	0.1 M Sodium HEPES pH 7.5		
C6	0.2 M Calcium chloride	0.1 M Sodium HEPES pH 7.5	28%(v/v) PEG 400	
C7	0.2 M Magnesium chloride	0.1 M Sodium HEPES pH 7.5	30%(v/v) Isopropanol	
C8		0.1M Imidazole pH 8.0	40% (v/v) PEG 400	
C9	10% (v/v) Glycerol	0.1 M HEPES pH 7.5	5% (w/v) PEG 3000,30% (v/v) PEG 400	
C10	0.2 M Sodium chloride	0.1 M Tris pH 7.0	1.0 M Sodium citrate	
C11		0.1 M Tris pH 7.0	15%(v/v) Ethanol	
C12	0.2 M Sodium chloride	0.1 M Tris pH 7.0	35%(v/v) MPD	
D1	0.2 M Sodium chloride	0.1M Imidazole pH 8.0	1.0 M Potassium/Sodium tartrate	
D2		0.1 M HEPES pH 6.5	40%(v/v) MPD	7,0
D3		0.1 M HEPES pH 6.5	20%(v/v) MPD	7,0
D4		1.0 M Imidazole pH 7.0		
D5	0.4 M Potassium/Sodium tartrate			
D6		0.1 M HEPES pH 6.5	2.4 M Ammonium sulfate	7,0
D7	1.0 M Lithium chloride	0.1 M HEPES pH 7.0	20%(w/v) PEG 6000	7,0
D8		0.1 M HEPES pH 6.5	5%(w/v) PEG 6000	7,0
D9		0.1 M Sodium cacodylate pH 6.5	35%(v/v) 2-Ethoxyethanol	
D10		0.1 M Tris pH 7.0	50% (v/v) PEG 200	
D11	0.2 M Sodium chloride	0.1 M Sodium/Potassium phosphate pH 6.2	35% (v/v) 2-Ethoxyethanol	
D12	1.0 M Sodium citrate	0.1 M Sodium cacodylate pH 6.5		
E1		0.1 M Sodium cacodylate pH 6.5	1.26 M Ammonium sulfate	
E2	0.01 M Cobalt chloride	0.1 M MES pH 6.5	1.8 M Ammonium sulfate	
E3		0.1 M MES pH 6.5	1.6 M Ammonium sulfate,10%(v/v) 1,4-Dioxane	
E4		0.1 M MES pH 6.5	1.6 M Magnesium sulfate	
E5	0.16 M Calcium acetate	0.08 M Sodium cacodylate pH 6.5	14.4%(w/v) PEG 8000, 20%(v/v) Glycerol	
E6	0.18 M Magnesium acetate	0.09 M Sodium cacodylate pH 6.5	27% (v/v) MPD,10% (v/v) Glycerol	
E7	0.16 M Magnesium acetate	0.08 M Sodium cacodylate pH 6.5	16%(w/v) PEG 8000, 20%(v/v) Glycerol	
E8	0.2 M Calcium acetate	0.1 M Sodium cacodylate pH 6.5	18%(w/v) PEG 8000	
E9	0.2 M Sodium acetate	0.1 M Sodium cacodylate pH 6.5	30%(w/v) PEG 8000	
E10		0.1 M Imidazole pH 6.5	1.0 M Sodium acetate	
E11	0.2 M Magnesium acetate	0.1 M Sodium cacodylate pH 6.5	30%(v/v) MPD	
E12		0.1 M Sodium cacodylate pH 6.5	1.4 M Sodium acetate	
F1		0.1M MES pH 6.0	40%(v/v) PEG 400, 5% (w/v) PEG 3000	

F2		0.1M Sodium citrate pH 5.5	35%(v/v) 2-Ethoxyethanol	
F3		0.1 M Sodium/Potassium phosphate pH 6.2	35%(v/v) MPD	
F4		0.1 M Sodium/Potassium phosphate pH 6.2	2.5 M Sodium chloride	
F5	0.2 M Calcium acetate	0.1 M MES pH 6.0	10%(v/v) Isopropanol	
F6	0.2 M Zinc acetate	0.1 M MES pH 6.0	10% (w/v) PEG 8000	
F7		0.1 M MES pH 6.0	3.2 M Ammonium sulfate	
F8		0.1 M MES pH 5.0	2.4 M Ammonium sulfate	6,0
F9		0.1 M MES pH 5.0	0.8 M Ammonium sulfate	6,0
F10	0.2 M Potassium/Sodium tartrate	0.1 M Sodium citrate pH 5.6	2.0 M Ammonium sulfate	
F11	0.17 M Ammonium acetate	0.085 M Sodium citrate pH 5.6	25.5%(w/v) PEG 4000, 15%(v/v) Glycerol	
F12		0.1 M Sodium citrate pH 5.6	1.0 M Ammonium dihydrogen phosphate	
G1		0.1 M Sodium citrate pH 5.5	2.0 M Ammonium sulfate	
G2		0.1M Sodium acetate pH 4.5	40% (v/v) PEG 400	
G3		0.1M Tris pH 7.0	40% (v/v) PEG 300, 5% (w/v) PEG 1000	
G4		0.1M Phosphate-citrate pH 4.2	40%(v/v) PEG 600	
G5	0.2 M Calcium chloride		20%(w/v) PEG 3350	
G6		0.1 M Sodium acetate pH 5.0	40%(v/v) MPD	5,0
G7		0.1 M Citric Acid pH 5.0	1.0 M Lithium chloride	5,0
G8		0.1 M Citric Acid pH 4.0	30%(w/v) PEG 6000	5,0
G9		0.04 M Potassium dihydrogen phosphate	16%(w/v) PEG 8000, 20%(v/v) Glycerol	
G10	0.1 M Cadmium chloride	0.1 M Sodium acetate pH 4.6	30%(v/v) PEG 400	
G11	0.2 M Sodium chloride	0.1 M Sodium acetate pH 4.6	30%(v/v) MPD	
G12	2.0 M Sodium chloride	0.1 M Sodium acetate pH 4.6		
H1	2.0 M Sodium formate	0.1 M Sodium acetate pH 4.6		
H2	0.2 M Calcium chloride	0.1 M Sodium acetate pH 4.6	20%(v/v) Isopropanol	
H3	0.2 M Lithium sulfate	0.1 M Sodium acetate pH 4.5	2.5 M Sodium chloride	
H4		0.1 M Sodium acetate pH 4.5	20%(v/v) Butanediol	
H5	0.2 M Sodium chloride	0.1 M Sodium acetate pH 4.5	1.26 M Ammonium sulfate	
H6		0.26 M Ammonium dihydrogen phosphate	35%(v/v) Glycerol	
H7		0.1 M Citric Acid pH 2.5	40%(v/v) MPD	4,0
H8		0.1 M Citric Acid pH 3.5	2.4 M Ammonium sulfate	4,0
H9		0.1 M Citric Acid pH 3.5	1.6 M Ammonium sulfate	4,0
H10	2.0 M Sodium chloride		10%(w/v) PEG 6000	
H11	0.2 M Ammonium sulfate		30%(w/v) PEG 4000	
H12	0.2 M Ammonium sulfate		30%(w/v) PEG 8000	

PEG and pH screening

Well	Buffer	Precipitant
A1	0,1M Tis pH 6	10% PEG 600
A2	0,1M Tis pH 6	10% PEG 2000
A3	0,1M Tis pH 6	10% PEG 3350
A4	0,1M Tis pH 6	10% PEG 6000
A5	0,1M Tis pH 6	10% PEG 8000
A6	0,1M Tis pH 6	10% PEG 10000
A7	0,1M Tis pH 6	10% PEG 20000
A8	0,1M Tis pH 6.5	10% PEG 600
A9	0,1M Tis pH 6.5	10% PEG 2000
A10	0,1M Tis pH 6.5	10% PEG 3350
A11	0,1M Tis pH 6.5	10% PEG 6000

A12	0,1M Tis pH 6.5	10% PEG 8000
B1	0,1M Tis pH 6.5	10% PEG 10000
B2	0,1M Tis pH 6.5	10% PEG 20000
B3	0,1M Tis pH 7	10% PEG 600
B4	0,1M Tis pH 7	10% PEG 2000
B5	0,1M Tis pH 7	10% PEG 3350
B6	0,1M Tis pH 7	10% PEG 6000
B7	0,1M Tis pH 7	10% PEG 8000
B8	0,1M Tis pH 7	10% PEG 10000
B9	0,1M Tis pH 7	10% PEG 20000
B10	0,1M Tis pH 7.5	10% PEG 600
B11	0,1M Tis pH 7.5	10% PEG 2000
B12	0,1M Tis pH 7.5	10% PEG 3350
C1	0,1M Tis pH 7.5	10% PEG 6000
C2	0,1M Tis pH 7.5	10% PEG 8000
C3	0,1M Tis pH 7.5	10% PEG 10000
C4	0,1M Tis pH 7.5	10% PEG 20000
C5	0,1M Tis pH 8	10% PEG 600
C6	0,1M Tis pH 8	10% PEG 2000
C7	0,1M Tis pH 8	10% PEG 3350
C8	0,1M Tis pH 8	10% PEG 6000
C9	0,1M Tis pH 8	10% PEG 8000
C10	0,1M Tis pH 8	10% PEG 10000
C11	0,1M Tis pH 8	10% PEG 20000
C12	0,1M Tis pH 8.5	10% PEG 600
D1	0,1M Tis pH 8.5	10% PEG 2000
D2	0,1M Tis pH 8.5	10% PEG 3350
D3	0,1M Tis pH 8.5	10% PEG 6000
D4	0,1M Tis pH 8.5	10% PEG 8000
D5	0,1M Tis pH 8.5	10% PEG 10000
D6	0,1M Tis pH 8.5	10% PEG 20000
D7	0,1M Tis pH 9	10% PEG 600
D8	0,1M Tis pH 9	10% PEG 2000
D9	0,1M Tis pH 9	10% PEG 3350
D10	0,1M Tis pH 9	10% PEG 6000
D11	0,1M Tis pH 9	10% PEG 8000
D12	0,1M Tis pH 9	10% PEG 10000
E1	0,1M Tis pH 9	10% PEG 20000
E2	0,1M Tis pH 9.5	10% PEG 600
E3	0,1M Tis pH 9.5	10% PEG 2000
E4	0,1M Tis pH 9.5	10% PEG 3350
E5	0,1M Tis pH 9.5	10% PEG 6000
E6	0,1M Tis pH 9.5	10% PEG 8000
E7	0,1M Tis pH 9.5	10% PEG 10000
E8	0,1M Tis pH 9.5	10% PEG 20000
E9	0,1M Tis pH 7.5	20% PEG 600
E10	0,1M Tis pH 7.5	20% PEG 2000
E11	0,1M Tis pH 7.5	20% PEG 3350
E12	0,1M Tis pH 7.5	20% PEG 6000
F1	0,1M Tis pH 7.5	20% PEG 8000
F2	0,1M Tis pH 7.5	20% PEG 10000
F3	0,1M Tis pH 7.5	20% PEG 20000

F4	0,1M Tis pH 8	20% PEG 3350
F5	0,1M Tis pH 8	20% PEG 6000
F6	0,1M Tis pH 8	20% PEG 8000
F7	0,1M Tis pH 8	20% PEG 10000
F8	0,1M Tis pH 8	20% PEG 20000

PEG and NaCl screening

Well	Buffer	Salt	Precipitant
A1	0,1M Tis pH 7.5	0,1M NaCl	10% PEG 600
A2	0,1M Tis pH 7.5	0,1M NaCl	10% PEG 2000
A3	0,1M Tis pH 7.5	0,1M NaCl	10% PEG 3350
A4	0,1M Tis pH 7.5	0,1M NaCl	10% PEG 6000
A5	0,1M Tis pH 7.5	0,1M NaCl	10% PEG 8000
A6	0,1M Tis pH 7.5	0,1M NaCl	10% PEG 10000
A7	0,1M Tis pH 7.5	0,1M NaCl	10% PEG 20000
A8	0,1M Tis pH 7.5	0,2M NaCl	10% PEG 600
A9	0,1M Tis pH 7.5	0,2M NaCl	10% PEG 2000
A10	0,1M Tis pH 7.5	0,2M NaCl	10% PEG 3350
A11	0,1M Tis pH 7.5	0,2M NaCl	10% PEG 6000
A12	0,1M Tis pH 7.5	0,2M NaCl	10% PEG 8000
B1	0,1M Tis pH 7.5	0,2M NaCl	10% PEG 10000
B2	0,1M Tis pH 7.5	0,2M NaCl	10% PEG 20000
B3	0,1M Tis pH 7.5	0,3M Nacl	10% PEG 600
B4	0,1M Tis pH 7.5	0,3M Nacl	10% PEG 2000
B5	0,1M Tis pH 7.5	0,3M Nacl	10% PEG 3350
B6	0,1M Tis pH 7.5	0,3M Nacl	10% PEG 6000
B7	0,1M Tis pH 7.5	0,3M Nacl	10% PEG 8000
B8	0,1M Tis pH 7.5	0,3M Nacl	10% PEG 10000
B9	0,1M Tis pH 7.5	0,3M Nacl	10% PEG 20000
B10	0,1M Tis pH 7.5	0,4M NaCl	10% PEG 600
B11	0,1M Tis pH 7.5	0,4M NaCl	10% PEG 2000
B12	0,1M Tis pH 7.5	0,4M NaCl	10% PEG 3350
C1	0,1M Tis pH 7.5	0,4M NaCl	10% PEG 6000
C2	0,1M Tis pH 7.5	0,4M NaCl	10% PEG 8000
C3	0,1M Tis pH 7.5	0,4M NaCl	10% PEG 10000
C4	0,1M Tis pH 7.5	0,4M NaCl	10% PEG 20000
C5	0,1M Tis pH 7.5	0,5M NaCl	10% PEG 600
C6	0,1M Tis pH 7.5	0,5M NaCl	10% PEG 2000
C7	0,1M Tis pH 7.5	0,5M NaCl	10% PEG 3350
C8	0,1M Tis pH 7.5	0,5M NaCl	10% PEG 6000
C9	0,1M Tis pH 7.5	0,5M NaCl	10% PEG 8000
C10	0,1M Tis pH 7.5	0,5M NaCl	10% PEG 10000
C11	0,1M Tis pH 7.5	0,5M NaCl	10% PEG 20000
C12	0,1M Tis pH 8	0,1M NaCl	10% PEG 3350
D1	0,1M Tis pH 8	0,1M NaCl	10% PEG 6000
D2	0,1M Tis pH 8	0,1M NaCl	10% PEG 8000
D3	0,1M Tis pH 8	0,1M NaCl	10% PEG 10000
D4	0,1M Tis pH 8	0,1M NaCl	10% PEG 20000
D5	0,1M Tis pH 8	0,2M NaCl	10% PEG 3350
D6	0,1M Tis pH 8	0,2M NaCl	10% PEG 6000
D7	0,1M Tis pH 8	0,2M NaCl	10% PEG 8000
D8	0,1M Tis pH 8	0,2M NaCl	10% PEG 10000

D9	0,1M Tis pH 8	0,2M NaCl	10% PEG 20000
D10	0,1M Tis pH 8	0,3M Nacl	10% PEG 3350
D11	0,1M Tis pH 8	0,3M Nacl	10% PEG 6000
D12	0,1M Tis pH 8	0,3M Nacl	10% PEG 8000
E1	0,1M Tis pH 8	0,3M Nacl	10% PEG 10000
E2	0,1M Tis pH 8	0,3M Nacl	10% PEG 20000
E3	0,1M Tis pH 8	0,4M NaCl	20% PEG 3350
E4	0,1M Tis pH 8	0,4M NaCl	20% PEG 6000
E5	0,1M Tis pH 8	0,4M NaCl	10% PEG 8000
E6	0,1M Tis pH 8	0,4M NaCl	10% PEG 10000
E7	0,1M Tis pH 8	0,4M NaCl	10% PEG 20000
E8	0,1M Tis pH 8	0,5M NaCl	20% PEG 3350
E9	0,1M Tis pH 8	0,5M NaCl	20% PEG 6000
E10	0,1M Tis pH 8	0,5M NaCl	10% PEG 8000
E11	0,1M Tis pH 8	0,5M NaCl	10% PEG 10000
E12	0,1M Tis pH 8	0,5M NaCl	10% PEG 20000
F1	0,1M Tis pH 7.5	None	None
F2	0,1M Tis pH 7.5	0,1M NaCl	None
F3	0,1M Tis pH 7.5	0,2M NaCl	None
F4	0,1M Tis pH 7.5	0,3M Nacl	None
F5	0,1M Tis pH 7.5	0,4M NaCl	None
F6	0,1M Tis pH 7.5	0,5M NaCl	None
F7	0,1M Tis pH 8	None	None
F8	0,1M Tis pH 8	0,1M NaCl	None
F9	0,1M Tis pH 8	0,2M NaCl	None
F10	0,1M Tis pH 8	0,3M Nacl	None
F11	0,1M Tis pH 8	0,4M NaCl	None
F12	0,1M Tis pH 8	0,5M NaCl	None
G1	0,1M Tis pH 8,5	None	None
G2	0,1M Tis pH 8,5	0,1M NaCl	None
G3	0,1M Tis pH 8,5	0,2M NaCl	None
G4	0,1M Tis pH 8,5	0,3M Nacl	None
G5	0,1M Tis pH 8,5	0,4M NaCl	None
G6	0,1M Tis pH 8,5	0,5M NaCl	None
G7	0,1M Tis pH 9	None	None
G8	0,1M Tis pH 9	0,1M NaCl	None
G9	0,1M Tis pH 9	0,2M NaCl	None
G10	0,1M Tis pH 9	0,3M Nacl	None
G11	0,1M Tis pH 9	0,4M NaCl	None
G12	0,1M Tis pH 9	0,5M NaCl	None

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Well	Salt and additives	Buffer
A1	0.33% w/v 1,5-Naphthalenedisulfonic acid disodium salt, 0.33% w/v 2,5-Pyridinedicarboxylic acid, 0.33% w/v 3,5-Dinitrosalicylic acid	0.02 M HEPES sodium pH 6.8
A2	0.25% w/v Benzidine, 0.25% w/v Nicotinamide, 0.25% w/v Pyromellitic acid, 0.25% w/v Sulfaguanidine	0.02 M HEPES sodium pH 6.8
A3	0.25% w/v Gly-gly, 0.25% w/v Gly-gly-gly, 0.25% w/v Gly-gly-gly-gly, 0.25% w/v Pentaglycine	0.02 M HEPES sodium pH 6.8
A4	0.25% w/v 3,5-Dinitrosalicylic acid, 0.25% w/v 4-Aminobenzoic acid, 0.25% w/v Salicylic acid, 0.25% w/v Trimesic acid	0.02 M HEPES sodium pH 6.8
A5	0.33% w/v 4-Nitrobenzoic acid, 0.33% w/v 5-Sulfosalicylic acid dihydrate, 0.33% w/v Naphthalene-1,3,6-trisulfonic acid trisodium salt hydrate	0.02 M HEPES sodium pH 6.8
A6	0.33% w/v 2,6-Naphthalenedisulfonic acid disodium salt, 0.33% w/v 2,7-Naphthalenedisulfonic acid disodium salt, 0.33% w/v Anthraquinone-2,6-disulfonic acid disodium salt	0.02 M HEPES sodium pH 6.8
A7	0.33% w/v 1,5-Naphthalenedisulfonic acid disodium salt, 0.33% w/v Naphthalene-1,3,6-trisulfonic acid trisodium salt hydrate, 0.33% w/v PIPES	0.02 M HEPES sodium pH 6.8

A8	0.25% w/v Sodium 1-pentanesulfonate monohydrate, 0.25% w/v 3,5-Dinitrosalicylic acid, 0.25% w/v 3-Aminosalicylic acid, 0.25% w/v Salicylamide	0.02 M HEPES sodium pH 6.8
A9	0.16% w/v L-Histidine, 0.16% w/v L-Isoleucine, 0.16% w/v L-Leucine, 0.16% w/v L-Phenylalanine, 0.16% w/v L-Tryptophan, 0.16% w/v L-Tyrosine	0.02 M HEPES sodium pH 6.8
A10	0.2% w/v D-(+)-Trehalose dihydrate, 0.2% w/v Guanidine hydrochloride, 0.2% w/v Phenol, 0.2% w/v Trimethylamine N-oxide dihydrate, 0.2% w/v Urea	0.02 M HEPES sodium pH 6.8
A11	0.33% w/v 2,5-Pyridinedicarboxylic acid, 0.33% w/v 4-Nitrobenzoic acid, 0.33% w/v Mellitic acid	0.02 M HEPES sodium pH 6.8
A12	0.25% w/v Benzidine, 0.25% w/v Phenylglyoxal monohydrate, 0.25% w/v Sulfaguanidine, 0.25% w/v Sulfanilamide	0.02 M HEPES sodium pH 6.8
B1	0.33% w/v Anthrone, 0.33% w/v Congo Red, 0.33% w/v N-(2-Acetamido)-2-aminoethanesulfonic acid	0.02 M HEPES sodium pH 6.8
B2	0.33% w/v 1,3,5-Pentanetricarboxylic acid, 0.33% w/v 5-Sulfosalicylic acid dihydrate, 0.33% w/v Trimesic acid	0.02 M HEPES sodium pH 6.8
B3	0.25% w/v 5-Sulfoisophthalic acid monosodium salt, 0.25% w/v Cystathionine, 0.25% w/v Dithioerythritol, 0.25% w/v L-Citrulline	0.02 M HEPES sodium pH 6.8
B4	3,5-Dinitrosalicylic acid, 0.33% w/v 3-Aminobenzenesulfonic acid, 0.33% w/v 5-Sulfosalicylic acid dihydrate	0.02 M HEPES sodium pH 6.8
B5	0.33% w/v 2,7-Naphthalenedisulfonic acid disodium salt, 0.33% w/v Azelaic acid, 0.33% w/v trans-Cinnamic acid	0.02 M HEPES sodium pH 6.8
B6	0.33% w/v 2,6-Naphthalenedisulfonic acid disodium salt, 0.33% w/v 2-Aminobenzenesulfonic acid, 0.33% w/v m-Benzenedisulfonic acid disodium salt	0.02 M HEPES sodium pH 6.8
B7	0.33% w/v 1,4-Cyclohexanedicarboxylic acid, 0.33% w/v 2,2'-Thiodiglycolic acid, 0.33% w/v 5-Sulfoisophthalic acid monosodium salt	0.02 M HEPES sodium pH 6.8
B8	0.33% w/v 3-Aminobenzoic acid, 0.33% w/v 3-Aminosalicylic acid, 0.33% w/v Salicylic acid	0.02 M HEPES sodium pH 6.8
B9	0.25% w/v Hexamminecobalt(III) chloride, 0.25% w/v Salicylamide, 0.25% w/v Sulfanilamide, 0.25% w/v Vanillic acid	0.02 M HEPES sodium pH 6.8
B10	0.25% w/v p-Coumaric acid, 0.25% w/v Phenylurea, 0.25% w/v Poly(3-hydroxybutyric acid), 0.25% w/v Sulfaguanidine	0.02 M HEPES sodium pH 6.8
B11	0.25% w/v 1,4-Cyclohexanedicarboxylic acid, 0.25% w/v Methylenediphosphonic acid, 0.25% w/v Sulfanilic acid	0.02 M HEPES sodium pH 6.8
B12	0.25% w/v D-Fructose 1,6-diphosphate trisodium salt octahydrate, 0.25% w/v D-Glucose 6-phosphate, 0.25% w/v L-O-Phosphoserine, 0.25% w/v O-Phospho-L-tyrosine	0.02 M HEPES sodium pH 6.8
C1	0.25% w/v Benzamidine hydrochloride, 0.25% w/v L-Carnitine hydrochloride, 0.25% w/v L-Cystine, 0.25% w/v L-Ornithine hydrochloride	0.02 M HEPES sodium pH 6.8
C2	0.33% w/v Caffeine, 0.33% w/v Dithioerythritol, 0.33% w/v L-Methionine	0.02 M HEPES sodium pH 6.8
C3	0.25% w/v Ala-ala, 0.25% w/v Ala-gly, 0.25% w/v Gly-gly-gly-gly, 0.25% w/v Leu-gly-gly	0.02 M HEPES sodium pH 6.8
C4	0.2% w/v Aspartame, 0.2% w/v Gly-asp, 0.2% w/v Gly-ser, 0.2% w/v Ser-tyr, 0.2% w/v Tyr-phe	0.02 M HEPES sodium pH 6.8
C5	0.16% w/v Ala-ala, 0.16% w/v Aspartame, 0.16% w/v Gly-tyr, 0.16% w/v Leu-gly-gly, 0.16% w/v Ser-Glu, 0.16% w/v Tyr-ala	0.02 M HEPES sodium pH 6.8
C6	0.33% w/v Gly-phe, 0.33% w/v Gly-tyr, 0.33% w/v Leu-gly-gly	0.02 M HEPES sodium pH 6.8
C7	0.16% w/v Ala-ala, 0.16% w/v Gly-asp, 0.16% w/v Gly-gly, 0.16% w/v Gly-phe, 0.16% w/v Gly-ser, 0.16% w/v Ser-tyr	0.02 M HEPES sodium pH 6.8
C8	0.05% w/v Glycine, 0.05% w/v L-(-)-Threonine, 0.05% w/v L-(+)-Lysine, 0.05% w/v L-Alanine, 0.05% w/v L-Arginine, 0.05% w/v L-Asparagine monohydrate, 0.05% w/v L-Aspartic acid, 0.05% w/v L-Glutamic acid, 0.05% w/v L-Glutamine, 0.05% w/v L-Histidine, 0.05% w/v L-Isoleucine, 0.05% w/v L-Leucine, 0.05% w/v L-Methionine, 0.05% w/v L-Phenylalanine, 0.05% w/v L-Proline, 0.05% w/v L-Serine, 0.05% w/v L-Tryptophan, 0.05% w/v L-Tyrosine, 0.05% w/v L-Valine	0.02 M HEPES sodium pH 6.8
C9	0.2% w/v D-(+)-Maltose monohydrate, 0.2% w/v D-(+)-Melibiose monohydrate, 0.2% w/v D-(+)-Raffinose pentahydrate, 0.2% w/v D-(+)-Trehalose dihydrate, 0.2% w/v Stachyose hydrate	0.02 M HEPES sodium pH 6.8
C10	0.16% w/v b-Cyclodextrin, 0.16% w/v D-(+)-Cellobiose, 0.16% w/v D-(+)-Maltotriose, 0.16% w/v D-(+)-Melezitose hydrate, 0.16% w/v D-(+)-Raffinose pentahydrate, 0.16% w/v Stachyose hydrate	0.02 M HEPES sodium pH 6.8
C11	0.16% w/v Azelaic acid, 0.16% w/v m-Benzenedisulfonic acid disodium salt, 0.16% w/v Mellitic acid, 0.16% w/v Pimelic acid, 0.16% w/v Pyromellitic acid, 0.16% w/v trans-Cinnamic acid	0.02 M HEPES sodium pH 6.8
C12	0.25% w/v 5-Sulfoisophthalic acid monosodium salt, 0.25% w/v Anthraquinone-2,6-disulfonic acid disodium salt 412.30 (anhyd), 0.25% w/v N-(2-acetamido)-2-aminoethanesulfonic acid, 0.25% w/v Tetrahydroxy-1,4-benzoquinone hydrate	0.02 M HEPES sodium pH 6.8
D1	0.25% w/v 1,3,5-Pentanetricarboxylic acid, 0.25% w/v 5-Sulfosalicylic acid dihydrate, 0.25% w/v o-Sulfobenzoic acid monoammonium salt, 0.25% w/v Sodium 4-aminosalicylate dihydrate	0.02 M HEPES sodium pH 6.8
D2	0.06 M CHAPS, 0.06 M HEPES, 0.06 M Tris, 0.25% w/v Hexamminecobalt(III) chloride	0.02 M HEPES sodium pH 6.8
D3	0.06 M MES monohydrate, 0.06 M PIPES, 0.33% w/v Hexamminecobalt(III) chloride	0.02 M HEPES sodium pH 6.8
D4	0.005 M Gadolinium(III) chloride hexahydrate, 0.005 M Samarium(III) chloride hexahydrate, 0.05 M Benzamidine hydrochloride, 0.25% w/v Salicin	0.02 M HEPES sodium pH 6.8
D5	0.004 M Calcium chloride dihydrate, 0.004 M Magnesium chloride hexahydrate, 0.004 M Manganese(II) chloride tetrahydrate, 0.004 M Zinc chloride	0.02 M HEPES sodium pH 6.8
D6	0.004 M Cadmium chloride hydrate, 0.004 M Cobalt(II) chloride hexahydrate, 0.004 M Copper(II) chloride dihydrate, 0.004 M Nickel(II) chloride hexahydrate	0.02 M HEPES sodium pH 6.8
D7	0.25% w/v 3,5-Dinitrosalicylic acid, 0.25% w/v 3-Indolebutyric acid, 0.25% w/v Naphthalene-1,3,6-trisulfonic acid trisodium salt hydrate, 0.25% w/v trans-1,2-Cyclohexanedicarboxylic acid	0.02 M HEPES sodium pH 6.8

D8	0.2% w/v Betaine anhydrous, 0.2% w/v L-Glutamic acid, 0.2% w/v L-Proline, 0.2% w/v Taurine, 0.2% w/v Trimethylamine N-oxide dihydrate	0.02 M HEPES sodium pH 6.8
D9	0.25% w/v 1,2-Diaminocyclohexane sulfate, 0.25% w/v 4-Nitrobenzoic acid, 0.25% w/v Cystamine dihydrochloride, 0.25% w/v Spermine	0.02 M HEPES sodium pH 6.8
D10	0.25% w/v 1,5-Naphthalenedisulfonic acid disodium salt, 0.25% w/v 2,7-Naphthalenedisulfonic acid disodium salt, 0.25% w/v 5-Sulfoisophthalic acid monosodium salt, 0.25% w/v Sulfanilic acid	0.02 M HEPES sodium pH 6.8
D11	0.25% w/v 2,6-Naphthalenedisulfonic acid disodium salt, 0.25% w/v 4-Aminobenzoic acid, 0.25% w/v 5-Sulfosalicylic acid dihydrate, 0.25% w/v Naphthalene-1,3,6-trisulfonic acid trisodium salt hydrate	0.02 M HEPES sodium pH 6.8
D12	0.2% w/v Rhenium(IV) oxide, 0.2% w/v Sodium bromide, 0.2% w/v Sodium nitrate, 0.2% w/v Sodium phosphate dibasic dihydrate, 0.2% w/v Sodium tetraborate decahydrate	0.02 M HEPES sodium pH 6.8
E1	0.2% w/v Caffeine, 0.2% w/v Cytosine, 0.2% w/v Gallic acid, 0.2% w/v Nicotinamide, 0.2% w/v Sodium pyrophosphate tetrabasic decahydrate	0.02 M HEPES sodium pH 6.8
E2	1% w/v Dextran sulfate sodium salt, 0.005% w/v Dextranase, 0.005% w/v a-Amylase	0.02 M HEPES sodium pH 6.8
E3	1% w/v Tryptone	0.02 M HEPES sodium pH 6.8
E4	1% w/v Protamine sulfate	0.02 M HEPES sodium pH 6.8
E5	0.005% w/v Deoxyribonuclease I, 0.5% w/v Deoxyribonucleic acid, 0.005% w/v Ribonuclease A, 0.5% w/v Ribonucleic acid	0.02 M HEPES sodium pH 6.8
E6	0.5% w/v Casein, 0.5% w/v Hemoglobin, 0.005% w/v Pepsin, 0.005% w/v Protease, 0.005% w/v Proteinase K, 0.005% w/v Trypsin	0.02 M HEPES sodium pH 6.8
E7	1% w/v Ovalbumin, 0.005% w/v Pepsin, 0.005% w/v Proteinase K, 0.005% w/v Trypsin	0.02 M HEPES sodium pH 6.8
E8	0.2% w/v D-Sorbitol, 0.2% w/v Glycerol, 0.2% w/v Glycine, 0.2% w/v myo-Inositol, 0.2% w/v Sarcosine	0.02 M HEPES sodium pH 6.8
E9	0.2% w/v 1,4-Diaminobutane, 0.2% w/v Cystamine dihydrochloride, 0.2% w/v Diloxanide furoate, 0.2% w/v Sarcosine, 0.2% w/v Spermine	0.02 M HEPES sodium pH 6.8
E10	1,2-Diaminocyclohexane sulfate, 0.25% w/v 1,8-Diaminooctane, 0.25% w/v Cadaverine, 0.25% w/v Spermine	0.02 M HEPES sodium pH 6.8
E11	0.2% w/v 1,2-Diaminocyclohexane sulfate, 0.2% w/v Diloxanide furoate, 0.2% w/v Fumaric acid, 0.2% w/v Spermine, 0.2% w/v Sulfaguanidine	0.02 M HEPES sodium pH 6.8
E12	0.2% w/v 1,4-Diaminobutane, 0.2% w/v 1,8-Diaminooctane, 0.2% w/v Cadaverine, 0.2% w/v Cystamine dihydrochloride, 0.2% w/v Spermidine	0.02 M HEPES sodium pH 6.8
F1	0.25% w/v Methylenediphosphonic acid, 0.25% w/v Phytic acid sodium salt hydrate, 0.25% w/v Sodium pyrophosphate tetrabasic decahydrate, 0.25% w/v Sodium triphosphate pentabasic	0.02 M HEPES sodium pH 6.8
F2	0.2% w/v D-Fructose 1,6-diphosphate trisodium salt octahydrate, 0.2% w/v Glycerol phosphate disodium salt hydrate, 0.2% w/v L-O-Phosphoserine, 0.2% w/v O-Phospho-L-tyrosine, 0.2% w/v Phytic acid sodium salt hydrate	0.02 M HEPES sodium pH 6.8
F3	0.16% w/v 4-Aminobutyric acid, 0.16% w/v 6-Aminohexanoic acid, 0.16% w/v L-(+)-Lysine, 0.16% w/v L-Ornithine hydrochloride, 0.16% w/v Taurine, 0.16% w/v b-Alanine	0.02 M HEPES sodium pH 6.8
F4	0.2% w/v L-Arginine, 0.2% w/v L-Canavanine, 0.2% w/v L-Carnitine hydrochloride, 0.2% w/v L-Citrulline, 0.2% w/v Taurine	0.02 M HEPES sodium pH 6.8
F5	0.2% w/v 1,2,3-Heptanetriol, 0.2% w/v 1,3-Propanediol, 0.2% w/v 1,4-Butanediol, 0.2% w/v 1,6-Hexanediol, 0.2% w/v Resorcinol	0.02 M HEPES sodium pH 6.8
F6	0.2% w/v (+/-)-2-Methyl-2,4-pentanediol, 0.2% w/v 1,2,3-Heptanetriol, 0.2% w/v Diethylenetriaminepentakis(methylphosphonic acid), 0.2% w/v D-Sorbitol, 0.2% w/v Glycerol	0.02 M HEPES sodium pH 6.8
F7	0.2% w/v Barbituric acid, 0.2% w/v Betaine anhydrous, 0.2% w/v Phloroglucinol, 0.2% w/v Resorcinol, 0.2% w/v Tetrahydroxy-1,4-benzoquinone hydrate	0.02 M HEPES sodium pH 6.8
F8	0.2% w/v 1,4-Butanediol, 0.2% w/v 1,6-Hexanediol, 0.2% w/v Diethylenetriaminepentakis(methylphosphonic acid), 0.2% w/v myo-Inositol, 0.2% w/v Phloroglucinol	0.02 M HEPES sodium pH 6.8
F9	0.2% w/v 6-Aminohexanoic acid, 0.2% w/v Benzamidine hydrochloride, 0.2% w/v Congo Red, 0.2% w/v Nicotinamide, 0.2% w/v Salicin	0.02 M HEPES sodium pH 6.8
F10	0.2% w/v Anthrone, 0.2% w/v Benzidine, 0.2% w/v N-(2-Acetamido)-2-aminoethanesulfonic acid, 0.2% w/v Phenylurea, 0.2% w/v b-Alanine	0.02 M HEPES sodium pH 6.8
F11	0.25% w/v Sodium 1-pentanesulfonate monohydrate, 0.25% w/v 4-Aminobutyric acid, 0.25% w/v Cytosine, 0.25% w/v Salicylamide	0.02 M HEPES sodium pH 6.8
F12	0.11% w/v Dodecanedioic acid, 0.11% w/v Fumaric acid, 0.11% w/v Glutaric acid, 0.11% w/v Hexadecanedioic acid, 0.11% w/v Maleic acid, 0.11% w/v Oxamic acid, 0.11% w/v Pimelic acid, 0.11% w/v Sebacic acid, 0.11% w/v Suberic acid	0.02 M HEPES sodium pH 6.8
G1	0.16% w/v 5-Sulfosalicylic acid dihydrate, 0.16% w/v Dodecanedioic acid, 0.16% w/v Hippuric acid, 0.16% w/v Mellitic acid, 0.16% w/v Oxalacetic acid, 0.16% w/v Suberic acid	0.02 M HEPES sodium pH 6.8
G2	0.2% w/v 2,2'-Thiodiglycolic acid, 0.2% w/v Adipic acid, 0.2% w/v Benzoic acid, 0.2% w/v Oxalic acid anhydrous, 0.2% w/v Terephthalic acid	0.02 M HEPES sodium pH 6.8
G3	0.25% w/v 2,2'-Thiodiglycolic acid, 0.25% w/v Azelaic acid, 0.25% w/v Mellitic acid, 0.25% w/v trans-Aconitic acid	0.02 M HEPES sodium pH 6.8
G4	0.16% w/v 3-Indolebutyric acid, 0.16% w/v Hexadecanedioic acid, 0.16% w/v Oxamic acid, 0.16% w/v Pyromellitic acid, 0.16% w/v Sebacic acid, 0.16% w/v Suberic acid	0.02 M HEPES sodium pH 6.8
G5	0.25% w/v 1,3,5-Pentanetricarboxylic acid, 0.25% w/v 4-Hydroxyphenylacetic acid, 0.25% w/v Benzoic acid, 0.25% w/v Poly(3-hydroxybutyric acid)	0.02 M HEPES sodium pH 6.8

G6	0.16% w/v Glutaric acid, 0.16% w/v Mellitic acid, 0.16% w/v Oxalic acid anhydrous, 0.16% w/v Pimelic acid, 0.16% w/v Sebacic acid, 0.16% w/v trans-Cinnamic acid	0.02 M HEPES sodium pH 6.8
G7	0.2% w/v 4-Aminobenzoic acid, 0.2% w/v Azelaic acid, 0.2% w/v o-Sulfobenzoic acid monoammonium salt, 0.2% w/v p-Coumaric acid, 0.2% w/v Sodium 4-aminosalicylate dihydrate	0.02 M HEPES sodium pH 6.8
G8	0.16% w/v 3-Aminobenzenesulfonic acid, 0.16% w/v 3-Aminobenzoic acid, 0.16% w/v Hippuric acid, 0.16% w/v Oxalacetic acid, 0.16% w/v Salicylic acid, 0.16% w/v Trimesic acid	0.02 M HEPES sodium pH 6.8
G9	0.2% w/v 2-Aminobenzenesulfonic acid, 0.2% w/v 3-Indolebutyric acid, 0.2% w/v 4-Hydroxyphenylacetic acid, 0.2% w/v Barbituric acid, 0.2% w/v Terephthalic acid	0.02 M HEPES sodium pH 6.8
G10	0.2% w/v 1,4-Cyclohexanedicarboxylic acid, 0.2% w/v 2,5-Pyridinedicarboxylic acid, 0.2% w/v Glutaric acid, 0.2% w/v trans-1,2-Cyclohexanedicarboxylic acid, 0.2% w/v trans-Aconitic acid	0.02 M HEPES sodium pH 6.8
G11	10% v/v Tacsimate pH 7.0	0.02 M HEPES sodium pH 6.8
G12	0.2% w/v Benzenephosphonic acid, 0.2% w/v Gallic acid, 0.2% w/v Melatonin, 0.2% w/v N-(2-carboxyethyl)-iminodiacetic acid, 0.2% w/v Trimellitic acid	0.02 M HEPES sodium pH 6.8
H1	0.05% w/v Glycine, 0.05% w/v L-(-)-Threonine, 0.05% w/v L-(+)-Lysine, 0.05% w/v L-Alanine, 0.05% w/v L-Arginine, 0.05% w/v L-Asparagine monohydrate, 0.05% w/v L-Aspartic acid, 0.05% w/v L-Glutamic acid, 0.05% w/v L-Glutamine, 0.05% w/v L-Histidine, 0.05% w/v L-Isoleucine, 0.05% w/v L-Leucine, 0.05% w/v L-Methionine, 0.05% w/v L-Phenylalanine, 0.05% w/v L-Proline, 0.05% w/v L-Serine, 0.05% w/v L-Tryptophan, 0.05% w/v L-Tyrosine, 0.05% w/v L-Valine	0.02 M HEPES sodium pH 6.8
H2	0.2% w/v Ala-ala, 0.2% w/v Ala-gly, 0.2% w/v Gly-asp, 0.2% w/v Gly-phe, 0.2% w/v Ser-Glu	0.02 M HEPES sodium pH 6.8
H3	0.2% w/v 3,5-Dinitrosalicylic acid, 0.2% w/v 4-Aminobenzoic acid, 0.2% w/v Benzamidine hydrochloride, 0.2% w/v Hexamminecobalt(III) chloride, 0.2% w/v Mellitic acid	0.02 M HEPES sodium pH 6.8
H4	0.16% w/v 1,4-Diaminobutane, 0.16% w/v 1,8-Diaminooctane, 0.16% w/v Cadaverine, 0.16% w/v Cystamine dihydrochloride, 0.16% w/v Spermidine, 0.16% w/v Spermine	0.02 M HEPES sodium pH 6.8
H5	0.16% w/v 4-Aminobutyric acid, 0.16% w/v 6-Aminohexanoic acid, 0.16% w/v Oxamic acid, 0.16% w/v Sulfanilic acid, 0.16% w/v Trimesic acid, 0.16% w/v b-Alanine	0.02 M HEPES sodium pH 6.8
H6	0.16% w/v D-3-Phosphoglyceric acid disodium salt, 0.16% w/v D-Fructose 1,6-diphosphate trisodium salt octahydrate, 0.16% w/v D-Glucose 6-phosphate, 0.16% w/v L-O-Phosphoserine, 0.16% w/v O-Phospho-L-tyrosine, 0.16% w/v Phytic acid sodium salt hydrate	0.02 M HEPES sodium pH 6.8
H7	0.0625% w/v 1,3,5-Pentanetricarboxylic acid, 0.0625% w/v Azelaic acid, 0.0625% w/v Dodecanedioic acid, 0.0625% w/v Glutaric acid, 0.0625% w/v Hexadecanedioic acid, 0.0625% w/v Pimelic acid, 0.0625% w/v Sebacic acid, 0.0625% w/v Suberic acid	0.02 M HEPES sodium pH 6.8
H8	0.16% w/v 1,5-Naphthalenedisulfonic acid disodium salt, 0.16% w/v 2,6-Naphthalenedisulfonic acid disodium salt, 0.16% w/v 2,7-Naphthalenedisulfonic acid disodium salt, 0.16% w/v 4-Nitrobenzoic acid, 0.16% w/v m-Benzenedisulfonic acid disodium salt, 0.16% w/v Naphthalene-1,3,6-trisulfonic acid trisodium salt hydrate	0.02 M HEPES sodium pH 6.8
H9	0.2% w/v 2,5-Pyridinedicarboxylic acid, 0.2% w/v Pyromellitic acid, 0.2% w/v Salicylic acid, 0.2% w/v trans-1,2-Cyclohexanedicarboxylic acid, 0.2% w/v trans-Cinnamic acid	0.02 M HEPES sodium pH 6.8
H10	0.16% w/v 3-Aminobenzenesulfonic acid, 0.16% w/v 5-Sulfosalicylic acid dihydrate, 0.16% w/v p-Coumaric acid, 0.16% w/v PIPES, 0.16% w/v Terephthalic acid, 0.16% w/v Vanillic acid	0.02 M HEPES sodium pH 6.8
H11	0.07% w/v Barbituric acid, 0.07% w/v Benzidine, 0.07% w/v Cystathionine, 0.07% w/v L-Canavanine, 0.07% w/v L-Carnitine hydrochloride, 0.07% w/v L-Cystine, 0.07% w/v Mellitic acid	0.02 M HEPES sodium pH 6.8
H12	0.16% w/v Aspartame, 0.16% w/v Gly-gly-gly, 0.16% w/v Leu-gly-gly, 0.16% w/v Pentaglycine, 0.16% w/v Tyr-ala, 0.16% w/v Tyr-phe	0.02 M HEPES sodium pH 6.8

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