

Morpheus® II 10 mL, HT-96 and FX-96 pre-filled plate. MD1-91, MD1-92 and MD1-92-FX

Morpheus II is optimized to yield crystals not observed in traditional screens and also includes heavy atoms for experimental phasing as well as polyols for cryoprotection.

MD1-91 is presented as 96 x 10 mL conditions *(includes 1 x 15 mL glass bottle containing polyamine powders¹ to mix with water)*. MD1-92 is presented as 96 x 1 mL conditions / MD1-92-FX is presented as 96 x 100 µL conditions.

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Features of Morpheus II:

- Targeted incorporation of 35 low- molecular weight PDB ligands into 96 conditions.
- Incorporation of heavy atoms as additives for experimental phasing.
- Suitable for membrane proteins with PEGS and polyols as main precipitants.
- The inclusion of NDSBs, polyamines, amino acids and monosaccharides to enhance solubility and stability of many proteins for crystallization.
- New polyols included for cryoprotection of conditions: no need to investigate more conditions for cryo-cooling.
- Innovative buffer systems facilitate pH optimization.
- Readily available Morpheus® II Optimization reagents including the Mixes and Stock reagents.

Introduction

Morpheus II is a follow up to the original screen that was used with success in many laboratories. Morpheus II integrates reagents that are not seen in other screens, especially less common additives. As a consequence, the screen should have an impact not only on crystallisation but also on the overall structure determination process. Morpheus II follows the general design principles of the original Morpheus screen. However, less typical additives have been included, such as heavy metals, NDSBs, polyamines, amino acids and monosaccharides. In addition, various polyols have been added for cryoprotection along with innovative buffer systems.

Some additives such as metal divalent cations can induce new crystal contacts. Also, heavy atoms were integrated to help with *ab initio* structure determination since a common issue to solve novel structure is the phase problem.

Most of the other additives are meant to alter protein stability and solubility (carboxylic acids, polyamines and monosaccharides).



Figure 1

Figure 1. Examples of crystals obtained during initial tests with the final formulation of Morpheus II. A. B. Concanavalin-A (MW = 27 kDa). C. Catalase (63 kDa). D. BAR domain (29 kDa). E. Trans-membrane complex (540 kDa). F. Actin homologue (37 kDa). G. Domain of a cytosolic nucleic acid sensor (12 kDa). H. Virus capsid (25 kDa). I. Polymerase complex (80 kDa).

¹ The mix of polyamines must be prepared and added to the 12 corresponding tubes by the user, hence only the raw chemicals are present in the screen kit (powder mix is given in a 15 ml glass bottle in order to prepare 13 ml using ultrapure water).

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Screen Design

Morpheus II is based on the 3D grid design of Morpheus. The 35 PDB-derived ligands selected to formulate Morpheus2 are shown in Table 1. From these, eight additive mixes were prepared (Table 2): LiNaK, Divalents II, Alkalis, Oxometaltes, Lanthanides, Monosaccharides II, Amino acids II and Polyamines.

Formulation Notes

Morpheus II reagents are formulated using ultrapure water (>18.0 M Ω) and are sterile-filtered using 0.22 μ m filters. No preservatives are added.

PLEASE NOTE

The polyamine mix must be prepared and added to the 12 corresponding tubes by the user. The raw chemicals in a powder mix are provided in the kit in a 15 mL glass bottle. Sufficient ultrapure (>18.0 M Ω) water should be added to this bottle to make up a 13 mL solution.

A light pellet may form in tubes 2-1 to 2-12/well numbers E-1 to E-12 which contain Lanthanides (2-1 to 2-12). It is easily re-suspended with gentle mixing. The screen should be kept between 10-18 °C and gently mixed before use.

Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Contact and product details can be found at <u>www.moleculardimensions.com</u>

Enquiries regarding Morpheus II formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

References

- 1. Gorrec, F (2009), The MORPHEUS protein crystallization screen *J Appl Cryst* 42, 1035-1042
- Gorrec, F (2013), The current approach to initial crystallization screening of proteins is under-sampled *J Appl Cryst* 46, 795-797.

Morpheus II Optimization

Although the screen is composed of various mixes, consider each condition as for any other screen, with three stock solutions:

- mix of precipitants
- mix of additives
- mix of buffers.

When you have more than one hit, you can deduce the importance of each stock from the beginning: e.g. Do I see specificity related to one stock? To pH?

Every condition can be made following the same ratio of stock solutions:

1/2 [Precipitant mix] + 1/10 [additive mix] + 1/10 [Buffer system] + 3/10 dH₂O.

To vary the pH, you can change the ratio of the two buffers within the buffer stock (i.e. change ratio of two non-titrated 1M buffer stocks).

Once you know more about the chemical space within Morpheus II you can eventually investigate further, trying to reveal specificity of a single chemical.

For example, what happens when you replace the group of chemicals from a stock with only one chemical of this mix? (e.g. only one divalent cations instead of the corresponding mix of additives).

At this stage you may (or not) have a simpler condition to work with. You can also proceed to other "classic" optimization approaches such as using an additive screen, scale-up or seeding.

Table 1: List of PDB ligands in Morpheus II

∕₽́DB ligand name	Ciass	PDB ID (main)	No. of structures*
Lithium sulfate	Common salt	LI	51
Sodium chloride	Common salt	NA	4726
Potassium sulfate	Common salt	К	1638
Manganese chloride tetrahydrate	Divalent cation	MN	1938
Cobalt chloride hexahydrate	Divalent cation	СО	474
Nickel chloride hexahydrate	Divalent cation	NI	699
Zinc acetate dihydrate	Divalent cation	ZN	8413
Barium acetate	Alkəli	BA	91
Cesium acetate	Alkali	CS	75
Rubidium chloride	Alkəli	RB	34
Strontium acetate	Alkali	SR	101
Sodium chromate tetrahydrate	Oxometalate	CR	7
Sodium molybdate dihydrate	Oxometalate	MOO	20
Sodium orthovanadate	Oxometalate	VO4	73
Sodium tungstate dihydrate	Oxometalate	WO4	47
Erbium (III) chloride hexahydrate	Lanthanide	ER3	2
Terbium (III) chloride hexahydrate	Lanthanide	ТВ	11
Ytterbium (III) chloride hexahydrate	Lanthanide	YB	57
Yttrium (III) chloride hexahydrate	Lanthanide	YT3	33
Xylitol	Monosaccharide	XYL	25
D-(-)-fructose	Monosaccharide	FRU; FUD	36; 4
D-sorbitol	Monosaccharide	SOR	12
Myo-inositol	Monosaccharide	INS	16
L-rhamnose monohydrate	Monosaccharide	RAM	43
DL-threonine	Amino-acid	DTH; THR	23;n/a
DL-histidine, HCI, H20	Amino-acid	DHI; HIS	24; n/a
DL-5-hydroxylysine, HCl	Amino-acid	n/a; LYZ	0; 7
Trans-4-hydroxy-L-proline	Amino-acid	HYP	149
Spermine, 4HCl	Polyamine	SPM	103
Spermidine, 3HCl	Polyamine	SPD	32
1,4-diaminobutane, 2HCl	Polyamine	PUT	22
DL-ornithine, HCl	Polyamine	ORD; ORN	3; 56
NDSB 256	Surfactant	DMX	4
NDSB 195	Surfactant	NDS	7
Bis-tris	Buffer	BTB	114

*No of structures as determined by a query of the pdb carried out in December 2014

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Table 2: Mixes of additives used in Morpheus II

Mix name	Composition	Catalogue Number (100 ml)	Catalogue Number (250 ml)
0.9 M LiNaK	0.3 M Lithium sulfate, 0.3 M Sodium sulfate, 0.3 M Potassium sulfate	MD2-100-231	MD2-250-231
0.02M Divalents II	0.005M Manganese(II) chloride tetrahydrate, 0.005M Cobalt(II) chloride hexahydrate , 0.005M Nickel(II) chloride hexahydrate, 0.005M Zinc acetate dihydrate	MD2-100-232	MD2-250-232
0.04 M Alkalis	0.01M Rubidium chloride, 0.01M Strontium acetate, 0.01M Cesium acetate, 0.01M Barium acetate	MD2-100-233	MD2-250-233
0.02 M Oxometalates	0.005M Sodium chromate tetrahydrate, 0.005M Sodium molybdate dihydrate, 0.005M Sodium tungstate dihydrate, 0.005M Sodium orthovanadate	MD2-100-234	MD2-250-234
0.02M Lanthanides	0.005M Yttrium(III) chloride hexahydrate, 0.005M Erbium(III) chloride hexahydrate, 0.005M Terbium(III) chloride hexahydrate, 0.005M Ytterbium(III) chloride hexahydrate	MD2-100-235	MD2-250-235
1M Monosaccharides II	0.2M Xylitol, 0.2M <i>Myo</i> -Inositol, 0.2M D-(-)- Fructose, 0.2M L-Rhamnose monohydrate, 0.2M D-Sorbitol	MD2-100-236	MD2-250-236
1M Amino acids II	0.2M DL-Arginine hydrochloride, 0.2M DL- Threonine, 0.2M DL-Histidine monohydrochloride monohydrate, 0.2M DL-5-Hydroxylysine hydrochloride, 0.2M <i>trans</i> -4-hydroxy-L-proline	MD2-100-237	MD2-250-237
0.4 M Polyamines (provided as powder for 10mL kits)*	0.1M Spermine tetrahydrochloride, 0.1M Spermidine trihydrochloride, 0.1M 1,4- Diaminobutane dihydrochloride, 0.1M DL- Ornithine monohydrochloride	MD2-100-238	MD2-250-238

Table 3: Buffer systems used in Morpheus II

(
Mix name*	Conc.	рН @ 20 ° С	Composition	Catalogue Number (100 ml)	Catalogue Number (250 ml)
Buffer System 4	1.0M	6.5	MOPSO, Bis-Tris	MD2-100-243	MD2-250-243
Buffer System 5	1.0M	7.5	BES, Triethanolamine (TEA)	MD2-100-244	MD2-250-244
Buffer System 6	1.0M	8.5	Gly-Gly, AMPD	MD2-100-245	MD2-250-245

*Buffer systems 1, 2 & 3 are allocated to the original Morpheus screen.

Table 4: Mixes of Precipitants used in Morpheus II

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Mix name*	Composition	Catalogue Number (100 ml)	Catalogue Number (250 ml)
72% Precipitant Mix 5	30% w/v PEG 3000, 40% v/v 1, 2, 4- Butanetriol, 2% w/v NDSB 256	MD2-100-239	MD2-250-239
65% Precipitant Mix 6	25% w/v PEG 4000, 40% w/v 1,2,6- Hexanetriol	MD2-100-240	MD2-250-240
	4)

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60%Precipitant Mix 7	20% w/v PEG 8000, 40% v/v 1,5- Pentanediol	MD2-100-24	MD2-250-241
62% Precipitant Mix 8	10% w/v PEG 20000, 50% w/v Trimethylpropane, 2% w/v NDSB 19	MD2-100-24	MD2-250-242
*precipitant	Mixes 1, 2, 3 & 4 are allocated to		rpheus screen.
	Code	Pack Size	Description
	MD1-91		Morpheus II
	MD1-92		Morpheus II HT-96
	MD1-92-FX		
	Other Morpheus screen		Thorpheos in tx 50 pre timeo prot
	MD1-46		Morpheus
	MD1-47		Morpheus HT-96
	MD1-47-FX		Morpheus FX-96 pre-filled plate
	MD1-93	48 x 100 µL	The Morpheus® Additive screen
RE-ORDERI	N G MD1-116	96 x 10 mL	Morpheus III
INFORMATI	O N MD1-117	96 x 1 mL	Morpheus III HT-96
	MD1-118	48 x 100 µL	Hippocrates [™] additive screen
	Green screens (contain	green fluorescer	nt dye – ideal for UV)
	MD1-46-GREEN	96 x 10 mL	Morpheus Green screen
	MD1-47-GREEN	96 x 1 mL	Morpheus HT-96 Green screen
	Combo Packs		
	MD1-76	192 x 10 mL	Power combo value pack (Morpheus + MIDASplus)
	MD1-76-HT	192 x 1 mL	Power combo value pack HT-96 (Morpheus + MIDASplus HT-96)
	Single reagents		· · · · · · · · · · · · · · · · · · ·
	MDSR-91-tube number	100 mL	Morpheus II single reagents
	MDSR-92-well number	100 mL	Morpheus II HT-96 single reagen

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Morpheus II 10 mL Morpheus II HT-96 Morpheus II FX-96 MD1-91 MD1-92 MD1-92-FX

Conditions 1-48 (Box 1) Conditions A1-D12

Screen ID	Well #	Conc.		Additives (PDB ligands)	Conc.		Buffer	ρH	Conc.		Precipitant
1-1	A1	90	mΜ	LiNaK	0.1	М	Buffer System 4	6.5	36	% v/v	Precipitant Mix 5
1-2	A2		mΜ	LiNaK	0.1		Buffer System 4	6.5		% v/v	Precipitant Mix 6
1-3	A3	90		LiNaK	0.1		Buffer System 4	6.5		% v/v	Precipitant Mix 7
1-4	A4		mΜ	LiNaK	0.1		Buffer System 4	6.5	31		Precipitant Mix 8
1-5	A5		mΜ	LiNaK	0.1	М	Buffer System 5	7.5	36	% v/v	Precipitant Mix 5
1-6	A6	-	mΜ	LiNaK	0.1		Buffer System 5	7.5	32.5		Precipitant Mix 6
1-7	A7		mΜ	LiNaK	0.1		Buffer System 5	7.5		% v/v	Precipitant Mix 7
1-8	A8		mΜ	LiNaK	0.1		Buffer System 5	7.5	31		Precipitant Mix 8
1-9	A9		mΜ	LiNaK	0.1		Buffer System 6	8.5		% v/v	Precipitant Mix 5
1-10	A10		mΜ	LiNaK	0.1		Buffer System 6	8.5	32.5		Precipitant Mix 6
1-11	A11		mΜ	LiNaK	0.1		Buffer System 6	8.5	30		Precipitant Mix 7
1-12	A12		mΜ	LiNaK	0.1		Buffer System 6	8.5	31		Precipitant Mix 8
1-13	B1		mΜ	Divalents II	0.1		Buffer System 4	6.5		% v/v	Precipitant Mix 5
1-14	B2	-	mΜ	Divalents II	0.1		Buffer System 4	6.5	32.5		Precipitant Mix 6
1-15	B3		mΜ	Divalents II	0.1		Buffer System 4	6.5		% v/v	Precipitant Mix 7
1-16	B4	-	mΜ	Divalents II	0.1		Buffer System 4	6.5	31		Precipitant Mix 8
1-17	B5		mΜ	Divalents II	0.1		none	0.0		% v/v	Precipitant Mix 5
1-18	B6	-	mΜ	Divalents II			none		32.5		Precipitant Mix 6
1-19	B7	-	mΜ	Divalents II			none			% v/v	Precipitant Mix 7
1-20	B8		mΜ	Divalents II			none		31		Precipitant Mix 8
1-21	B9		mΜ	Divalents II	0.1	м	Buffer System 6	8.5		% v/v	Precipitant Mix 5
1-22	B10		mΜ	Divalents II	0.1		Buffer System 6	8.5	32.5		Precipitant Mix 6
1-23	B10		mΜ	Divalents II	0.1		Buffer System 6	8.5	30		Precipitant Mix 7
1-24	B12		mΜ	Divalents II	0.1		Buffer System 6	8.5	31		Precipitant Mix 8
1-25	C1		mΜ	Alkalis	0.1		Buffer System 4	6.5		% v/v	Precipitant Mix 5
1-26	C2		mΜ	Alkalis	0.1		Buffer System 4	6.5	32.5		Precipitant Mix 6
1-27	C3	-	mМ	Alkalis	0.1		Buffer System 4	6.5		% v/v	Precipitant Mix 7
1-28	C4			Alkalis	0.1		Buffer System 4	6.5	31		Precipitant Mix 8
1-29	C5			Alkalis	0.1		Buffer System 5	7.5		% v/v	Precipitant Mix 5
1-30	C6	-	mΜ	Alkalis	0.1		Buffer System 5	7.5	32.5		Precipitant Mix 6
1-31	C7	1	mΜ	Alkalis	0.1		Buffer System 5	7.5	30		Precipitant Mix 7
1-32	C8		mΜ	Alkalis	0.1		Buffer System 5	7.5	31		Precipitant Mix 8
1-33	C9	-	mΜ	Alkalis	0.1		Buffer System 6	8.5		% v/v	Precipitant Mix 5
1-34	C10		mΜ	Alkalis	0.1		Buffer System 6	8.5		% v/v	Precipitant Mix 6
1-35	C10		mM	Alkalis	0.1		Buffer System 6	8.5		% v/v	Precipitant Mix 7
1-36	C12	4		Alkalis	0.1		Buffer System 6	8.5	31	% v/v	Precipitant Mix 8
1-37	D1		mM	Oxometalates	0.1		Buffer System 4	6.5		% v/v	
1-37	D2	1	mM		-						Precipitant Mix 5
1-39	D2 D3			Oxometalates Oxometalates	0.1		Buffer System 4	6.5			Precipitant Mix 6
			mΜ		0.1		Buffer System 4	6.5		% v/v	Precipitant Mix 7
1-40	D4		mΜ	Oxometalates	0.1		Buffer System 4	6.5		% v/v	Precipitant Mix 8
1-41	D5	-	mΜ	Oxometalates	0.1		Buffer System 5	7.5		% v/v	
1-42	D6		mΜ	Oxometalates	0.1		Buffer System 5	7.5		% v/v	Precipitant Mix 6
1-43	D7		mΜ	Oxometalates	0.1		Buffer System 5	7.5		% v/v	Precipitant Mix 7
1-44	D8		mΜ	Oxometalates	0.1		Buffer System 5	7.5		% v/v	Precipitant Mix 8
1-45	D9		mΜ	Oxometalates	0.1		Buffer System 6	8.5		% v/v	Precipitant Mix 5
1-46	D10		mΜ	Oxometalates	0.1		Buffer System 6	8.5		% v/v	Precipitant Mix 6
1-47	D11		mΜ	Oxometalates	0.1		Buffer System 6	8.5		% v/v	Precipitant Mix 7
1-48	D12	2	mΜ	Oxometalates	0.1	M	Buffer System 6	8.5	31	% v/v	Precipitant Mix 8

Screen should be stored between 10-18°C and gently mixed before use



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Morpheus II 10 mL Morpheus II HT-96 Morpheus II FX-96 MD1-91 MD1-92 MD1-92-FX

Conditions 49-96 (Box 2) Conditions E1-H12

C	\A (= 11	6			C		Duffer		C	Desident	
Screen ID				Additives (PDB ligands)	Conc.		Buffer	ρH	Conc.	Precipitant	-
2-1	E1		mΜ	Lanthanides*	0.1		Buffer System 4	6.5	36 % v,		
2-2	E2		mΜ	Lanthanides*	0.1		Buffer System 4	6.5	32.5 % v,	· · ·	
2-3	E3		mΜ	Lanthanides*	0.1		Buffer System 4	6.5	30 % v,	·	
2-4	E4		mΜ	Lanthanides*	0.1		Buffer System 4	6.5	31 % v,		
2-5	E5	2	mΜ	Lanthanides*	0.1		Buffer System 5	7.5	36 % v,		5
2-6	E6	2	mΜ	Lanthanides*	0.1	Μ	Buffer System 5	7.5	32.5 % v,		6
2-7	E7	2	mΜ	Lanthanides*	0.1	Μ	Buffer System 5	7.5	30 % v,		7
2-8	E8	2	mΜ	Lanthanides*	0.1	Μ	Buffer System 5	7.5	31 % v,	v Precipitant Mix	8
2-9	E9	2	mΜ	Lanthanides*	0.1	Μ	Buffer System 6	8.5	36 % v,		5
2-10	E10	2	mΜ	Lanthanides*	0.1	Μ	Buffer System 6	8.5	32.5 % v,		6
2-11	E11	2	mΜ	Lanthanides*	0.1	Μ	Buffer System 6	8.5	30 % v,	v Precipitant Mix	7
2-12	E12	2	mΜ	Lanthanides*	0.1	Μ	Buffer System 6	8.5	31 % v,	v Precipitant Mix	8
2-13	F1	100	mΜ	Monosaccharides II	0.1	Μ	Buffer System 4	6.5	36 % v,	v Precipitant Mix	5
2-14	F2	100	mΜ	Monosaccharides II	0.1	Μ	Buffer System 4	6.5	32.5 % v,	v Precipitant Mix	6
2-15	F3	100	mΜ	Monosaccharides II	0.1	Μ	Buffer System 4	6.5	30 % v,	v Precipitant Mix	7
2-16	F4	100	mΜ	Monosaccharides II	0.1	Μ	Buffer System 4	6.5	31 % v,	v Precipitant Mix	8
2-17	F5	100	mΜ	Monosaccharides II	0.1	Μ	Buffer System 5	7.5	36 % v,	v Precipitant Mix	5
2-18	F6	100	mΜ	Monosaccharides II	0.1	Μ	Buffer System 5	7.5	32.5 % v	v Precipitant Mix	6
2-19	F7	100	mΜ	Monosaccharides II	0.1	М	Buffer System 5	7.5	30 % v	v Precipitant Mix	7
2-20	F8	100	mΜ	Monosaccharides II	0.1		Buffer System 5	7.5	31 % v,		
2-21	F9	100	mΜ	Monosaccharides II	0.1	М	Buffer System 6	8.5	36 % v,	· · ·	5
2-22	F10	100	mΜ	Monosaccharides II	0.1	_	Buffer System 6	8.5	32.5 % v		
2-23	F11	100		Monosaccharides II	0.1		Buffer System 6	8.5	30 % v,		
2-24	F12	100		Monosaccharides II	0.1		Buffer System 6	8.5	31 % v		
2-25	G1	100		Amino acids II	0.1		Buffer System 4	6.5	36 % v,		
2-26	G2	100		Amino acids II	0.1	_	Buffer System 4	6.5	32.5 % v		
2-27	G3	100		Amino acids II	0.1	_	Buffer System 4	6.5	30 % v	·	
2-28	G4	100		Amino acids II	0.1		Buffer System 4	6.5	31 % v		
2-29	G5	100		Amino acids II	0.1	_	Buffer System 5	7.5	36 % v		
2-30	G6	100		Amino acids II	0.1		Buffer System 5	7.5	32.5 % v		
2-31	G7	100		Amino acids II	0.1		Buffer System 5	7.5	30 % v		
2-32	G8	100		Amino acids II	0.1		Buffer System 5	7.5	31 % v		
2-33	G9	100		Amino acids II	0.1		Buffer System 6	8.5	36 % v		
2-34	G10	100		Amino acids II	0.1		Buffer System 6	8.5	32.5 % v	· · · ·	
2-35	G10 G11	100		Amino acids II	0.1		Buffer System 6	8.5	30 % v		
2-36	G12	100		Amino acids II	0.1		Buffer System 6	8.5	31 % v		
2-37	H1		mΜ	Polyamines†	0.1		Buffer System 4	6.5	36 % v		
2-37	H2		mM	Polyamines*	0.1		Buffer System 4	6.5	32.5 % v		
2-30	H3		mM	Polyamines†	0.1	_	Buffer System 4	6.5	30 % v	-	
2-39	H4		mΜ	Polyamines†	0.1			6.5		v Precipitant Mix	
2-41	H5		mΜ	Polyamines†	0.1		Buffer System 5	7.5	36 % v,		
2-42	H6		mΜ	Polyamines†	0.1	_	Buffer System 5	7.5	32.5 % v,		
2-43	H7		mM	Polyamines†	0.1		Buffer System 5	7.5	30 % v,		
2-44	H8		mΜ	Polyamines†	0.1		Buffer System 5	7.5	31 % v,		
2-45	H9		mΜ	Polyamines†	0.1		Buffer System 6	8.5	36 % v,		
2-46	H10		mΜ	Polyamines†	0.1		Buffer System 6	8.5	32.5 % v,		
2-47	H11		mΜ	Polyamines†	0.1		Buffer System 6	8.5	30 % v,		
2-48	H12	40	mΜ	Polyamines†	0.1	M	Buffer System 6	8.5	31 % v,	v Precipitant Mix	8

Screen should be stored between 10-18°C and gently mixed before use

*A light pellet may form in tubes 2-1 to 2-12/well numbers E-1 to E-12 which contain Lanthanides (2-1 to 2-12). It is easily re-suspended with gentle mixing.

[†]The polyamine mix must be prepared and added to the 12 corresponding tubes by the user. The raw chemicals in a powder mix are provided in the kit in a 15 mL glass bottle. Sufficient ultrapure (>18.0 M Ω) water should be added to this bottle to make up a 13 mL solution.