

Crystal Screen Lite™ is a complete reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules. Crystal Screen Lite is a straightforward, effective, and practical kit for determining preliminary crystallization conditions. Crystal Screen Lite is also effective in determining the solubility of a macromolecule in a wide range of precipitants and pH.

Crystal Screen Lite is a sparse matrix of trial crystallization reagent conditions based upon the original Jancarik and Kim screen.³ The primary screen variables are salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics). Crystal Screen Lite differs from the original Crystal Screen™ kit in that Crystal Screen Lite is formulated such that the primary precipitant reagents are one-half the concentration of that used in the original Crystal Screen formulation. The secondary salts, ions, and buffers remain at the original Crystal Screen concentration. Reducing the primary concentration of the primary precipitant results in a screen which is “more gentle” on the sample and typically produces much less precipitate conditions than the original Crystal Screen. Results comparing the Crystal Screen Lite formulation versus simply diluting the Crystal Screen formulation two-fold demonstrated more crystals using the Crystal Screen Lite protocol than the two-fold diluted Crystal Screen illustrating the importance of retaining the original salt, ion, and buffer concentration in Crystal Screen.⁵ Results comparing simply diluting the sample versus using Crystal Screen Lite also demonstrated more crystals when using Crystal Screen Lite than when simply diluting the sample. Crystal Screen Lite should be used with samples which demonstrate limited solubility in traditional crystallization reagents.

Sample Preparation

The macromolecular sample should be homogenous, as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or micro-filtration prior to use.^{1,2,4}

The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Crystal Screen Lite variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against water although ligands, ions, reducing agents, or other additives may be present as required by the sample for solubility, stability, or activity.

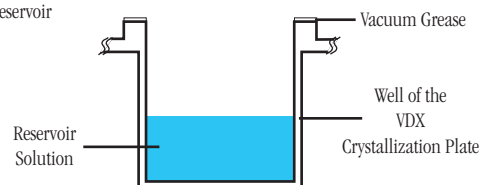
Performing The Screen

Since it is the most frequently reported method of crystallization, the following procedure describes the use of Crystal Screen Lite with the Hanging Drop Vapor Diffusion method. Crystal Screen Lite is also very compatible with the Sitting Drop, Sandwich Drop, Microbatch, and Microdialysis methods. A complete description of the Hanging, Sitting, Sandwich Drop, Dialysis and other crystallization methods are available from the Hampton Research Crystal Growth 101 Library.

1. Prepare a VDX Plate (HR3-140) for Hanging Drop Vapor Diffusion by applying a thin bead of cover slide sealant to the upper edge of each of the 24 reservoirs. One may also use a Greased VDX Plate (HR3-170). Fifty reservoirs are to be prepared for a complete Crystal Screen Lite. See Figure 1.

Figure 1

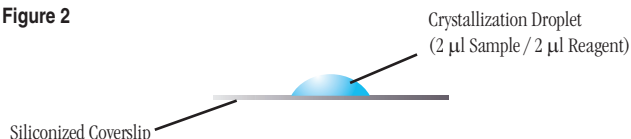
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Crystal Screen Lite reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of Crystal Screen Lite reagent 2 into reservoir A2. Repeat the procedure for the remaining 48 Crystal Screen Lite reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

3. Pipet 2 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

Figure 2

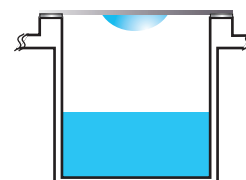


4. Pipet 2 µl of Crystal Screen Lite reagent 1 from reservoir A1 into the sample droplet and mix by aspirating and dispensing the droplet several times, keeping the tip in the drop during mixing to avoid foaming. See Figure 2.

5. Working quickly to minimize evaporation, invert the cover slide and droplet over reservoir A1 and seal the cover slide onto the edge of the reservoir. See Figure 3.

Figure 3

Inverted siliconized coverslip placed over the reservoir.



6. Repeat operations 3 through 5 for the remaining 49 Crystal Screen Lite reagents.

7. If the quantity of sample permits, perform Crystal Screen Lite in duplicate and incubate one set of plates at 4°C and the second set at room temperature. Incubate and store the crystallization plates in a stable temperature environment free of vibration.

How to Reproduce Crystal Screen Lite Reagents

Crystal Screen Lite reagents and optimization conditions based on Crystal Screen Lite hits can be formulated using volumetric methods and carefully prepared reagent stocks (Table 1). Note the examples below.

Example 1. To prepare 1.0 milliliter of Crystal Screen Lite reagent 1 in a crystallization plate.

Solution Composition: 0.02 M Calcium chloride dihydrate
0.1 M Sodium acetate trihydrate pH 4.6
15 % v/v (+/-)-2-Methyl-2,4-pentanediol

- 730 µl water³
- 20 µl 2.0 M Calcium chloride dihydrate (CAS # 10035-04-8, Catalog # HR2-557)
- 100 µl 1.0 M Sodium acetate trihydrate pH 4.6 (CAS # 6131-90-4, Catalog # HR2-731)
- 150 µl 100 % (+/-)-2-Methyl-2,4-pentanediol (CAS # 107-41-5, Catalog # HR2-627)

Make no pH adjustments. Mix well by aspirating and dispensing the solution multiple times.

Example 2. To prepare 1.0 milliliter of Crystal Screen Lite reagent 6.

Solution Composition: 0.2 M Magnesium chloride hexahydrate
0.1 M TRIS hydrochloride pH 8.5
15 % w/v Polyethylene glycol 4,000

- 500 µl water³
- 100 µl 1.0 M TRIS hydrochloride pH 8.5 (CAS # 1185-53-1, Catalog # HR2-727)
- 100 µl 2.0 M Magnesium chloride hexahydrate (CAS # 7791-18-6, Catalog # HR2-555)
- 300 µl 50% w/v Polyethylene glycol 4,000 (CAS # 25322-68-3, Catalog # HR2-529)

Make no pH adjustments. Mix well.

Example 3. To prepare 10 milliliters of Crystal Screen Lite reagent 8.

Solution Composition: 0.2 M Sodium citrate tribasic dihydrate
0.1 M Sodium cacodylate trihydrate pH 6.5
15 % v/v 2-Propanol

- 6,250 µl water³
- 1,000 µl 1.0 M Sodium cacodylate trihydrate pH 6.5 (CAS # 6131-99-3, Catalog # HR2-737)
- 1,250 µl 1.6 M Sodium citrate tribasic dihydrate (CAS # 6132-04-3, Catalog # HR2-549)
- 1,500 µl 100% 2-Propanol (CAS # 67-63-0, Catalog # HR2-619)

Make no pH adjustments. Mix well. Cover to prevent evaporation of the 2-Propanol.

³ ASTM Type II (laboratory grade) or Type III (analytical grade) water.

Formulation Notes for Crystal Screen Lite Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce a Crystal Screen Lite reagent.
2. All Optimize solutions and screen reagents are sterile filtered using 0.22 µm filters into sterile containers.
3. Add water first as this will help maintain the solubility of subsequently added reagents.
4. When formulating reagents using a pipet, add the largest volume last (except water). Use this larger volume setting to aspirate and dispense the reagent until the solution is mixed.
5. When formulating reagents using a pipet, use a clean, sterile pipet tip for each reagent added to the solution.
6. Use the buffers in Table 2 to systematically vary the pH as a crystallization variable.

pH as a Crystallization Variable

The buffers listed in Table 2, can be used to vary the pH as a crystallization variable and are recommended when optimizing a crystal grown from a Crystal Screen Lite kit.

Optimize™ buffer stocks are supplied as a 100 milliliters sterile filtered solution. The pH can be adjusted to the indicated pH range using either HCl or NaOH and the supplied titration tables.

StockOptions™ buffer kits contain 10 milliliters each of ready to pipet buffers, titrated in 0.1 pH increments over the indicated pH range. The number of reagents offered in a StockOptions buffer kit depends upon the pH range of the buffer. The broader the pH range, the more buffers in the kit.

Online Information

Visit www.hamptonresearch.com and enter one of the following:

- Reagent Catalog Number
- Kit Catalog Number
- CAS Number
- Reagent Name

To obtain reagent specifications, pH titration tables, user guides, certificates of analysis, material safety data sheets (MSDS), and any other additional information.

Crystal Screen Lite™

MakeTray™

MakeTray is a free, web based program at www.hamptonresearch.com which generates both a pipetting worksheet and a reagent formulation document for crystallization set ups. MakeTray allows one to enter general information about the sample and experiment, which is then

printed on the pipet worksheet and the reagent formulation document. The plate size can be customized for any number of wells, so MakeTray works for: 24, 48, and 96 well plates. MakeTray is especially useful for the design and formulation of crystal optimization experiments.

Table 1. Recommended reagents for the formulation of Crystal Screen Lite and optimization reagents.

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 below provides the common chemical name, the Hampton Research catalog number, supplied stock concentration, the supplied volume, and the CAS number for each reagent. For more information on a specific Optimize reagent, go to

www.hamptonresearch.com. Using Search, enter either the catalog number, CAS number, or chemical name to obtain additional information for the Optimize reagent, including a Certificate of Analysis and MSDS (where applicable).

Salts	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Ammonium acetate	HR2-565	1.0 M	100 ml	631-61-8
	HR2-799	8.0 M	200 ml	631-61-8
Ammonium phosphate monobasic	HR2-555	2.5 M	200 ml	7722-76-1
Ammonium sulfate	HR2-541	3.5 M	200 ml	7783-20-2
Calcium acetate hydrate	HR2-567	1.0 M	100 ml	62-54-4
Calcium chloride dihydrate	HR2-557	2.0 M	100 ml	10035-04-8
Lithium sulfate monohydrate	HR2-545	2.0 M	200 ml	10377-48-7
Magnesium acetate tetrahydrate	HR2-561	1.0 M	100 ml	16674-78-5
Magnesium chloride hexahydrate	HR2-559	2.0 M	100 ml	7791-18-6
	HR2-803	5.0 M	200 ml	7791-18-6
Magnesium formate dihydrate	HR2-537	1.0 M	200 ml	557-39-1
Potassium phosphate monobasic	HR2-553	1.5 M	200 ml	7778-77-0
Potassium sodium tartrate tetrahydrate	HR2-539	1.5 M	200 ml	6381-59-5
Sodium acetate trihydrate	HR2-543	3.0 M	200 ml	6131-90-4
Sodium citrate tribasic dihydrate	HR2-549	1.6 M	200 ml	6132-04-3
Sodium formate	HR2-547	7.0 M	200 ml	141-53-7
Sodium phosphate monobasic monohydrate	HR2-551	4.0 M	200 ml	10049-21-5
Zinc acetate dihydrate	HR2-563	1.0 M	100 ml	5970-45-6
Polymers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Polyethylene glycol 400	HR2-603	100 %	200 ml	25322-68-3
Polyethylene glycol 1,500	HR2-525	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 4,000	HR2-529	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 8,000	HR2-535	50 % w/v	200 ml	25322-68-3

(Continued on page 3)

Table 1 (Continued). Recommended reagents for the formulation of Crystal Screen Lite and optimization reagents.

Organics (volatile)	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
2-Propanol	HR2-619	100 %	200 ml	67-63-0
Organics (non-volatile)	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100 %	200 ml	107-41-5
Buffers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
HEPES sodium pH 7.5 ¹	HR2-733	1.0 M	100 ml	75277-39-3
Imidazole	HR2-573	1.0 M	100 ml	288-32-4
Sodium acetate trihydrate pH 4.6 ¹	HR2-731	1.0 M	100 ml	6131-90-4
Sodium cacodylate trihydrate pH 6.5 ¹	HR2-737	1.0 M	100 ml	6131-99-3
Sodium citrate tribasic dihydrate pH 5.6 ¹	HR2-735	1.0 M	100 ml	6132-04-3
TRIS hydrochloride pH 8.5 ²	HR2-727	1.0 M	100 ml	1185-53-1
¹ pH titrated using Hydrochloric acid (HR2-581) CAS # 7647-01-0				
² pH titrated using Sodium hydroxide (HR2-583) CAS # 1310-73-2				

Table 2. Recommended buffers for screening the pH of Crystal Screen Lite and optimization reagents.

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
Hepes sodium <u>untitrated</u>	HR2-577	1.0 M	100 ml	75277-39-3	6.6 - 8.5
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Hepes kit ⁴	HR2-231	1.0 M	10 ml each	75277-39-3	6.8 - 8.2
Imidazole <u>untitrated</u>	HR2-573	1.0 M	100 ml	288-32-4	6.2 - 7.8
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
Sodium acetate trihydrate <u>untitrated</u>	HR2-569	1.0 M	100 ml	6131-90-4	3.6 - 5.6
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-07	—
StockOptions™ Sodium Acetate kit ⁴	HR2-233	1.0 M	10 ml each	6131-90-4	3.6 - 5.6
Sodium cacodylate trihydrate <u>untitrated</u>	HR2-575	1.0 M	100 ml	6131-99-3	5.0 - 7.4
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Cacodylate kit ⁴	HR2-239	1.0 M	10 ml each	6131-99-3	5.1 - 7.4

(Continued on page 4)

Crystal Screen Lite™



Crystal Screen Lite Fundamentals

HR2-128 (pg 4)

Buffer Solution <u>or</u> Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
Sodium citrate tribasic dihydrate <u>untitrated</u>	HR2-571	1.0 M	100 ml	6132-04-3	3.0 - 6.2
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Citrate kit ⁴	HR2-235	1.0 M	10 ml each	6132-04-3	4.2 - 6.5
TRIS hydrochloride <u>untitrated</u>	HR2-579	1.0 M	100 ml	1185-53-1	7.0 - 9.0
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ TRIS hydrochloride kit ⁴	HR2-237	1.0 M	10 ml each	1185-53-1	7.0 - 9.0
⁴ Individual StockOptions buffers titrated to any pH within the kit's pH range are available in 185 ml volumes from the Hampton Research Custom Shop					

Technical Support

Inquiries regarding Crystal Screen Lite Fundamentals, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 5:00 p.m. USA Pacific Standard Time.

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Tube #	Salt	Tube #	Buffer ◇	Tube #	Precipitant
1.	0.02 M Calcium chloride dihydrate	1.	0.1 M Sodium acetate trihydrate pH 4.6	1.	15% v/v (+/-)-2-Methyl-2,4-pentanediol
2.	None	2.	None	2.	0.2 M Potassium sodium tartrate tetrahydrate
3.	None	3.	None	3.	0.2 M Ammonium phosphate monobasic
4.	None	4.	0.1 M TRIS hydrochloride pH 8.5	4.	1.0 M Ammonium sulfate
5.	0.2 M Sodium citrate tribasic dihydrate	5.	0.1 M HEPES sodium pH 7.5	5.	15% v/v (+/-)-2-Methyl-2,4-pentanediol
6.	0.2 M Magnesium chloride hexahydrate	6.	0.1 M TRIS hydrochloride pH 8.5	6.	15% w/v Polyethylene glycol 4,000
7.	None	7.	0.1 M Sodium cacodylate trihydrate pH 6.5	7.	0.7 M Sodium acetate trihydrate
8.	0.2 M Sodium citrate tribasic dihydrate	8.	0.1 M Sodium cacodylate trihydrate pH 6.5	8.	15% v/v 2-Propanol
9.	0.2 M Ammonium acetate	9.	0.1 M Sodium citrate tribasic dihydrate pH 5.6	9.	15% w/v Polyethylene glycol 4,000
10.	0.2 M Ammonium acetate	10.	0.1 M Sodium acetate trihydrate pH 4.6	10.	15% w/v Polyethylene glycol 4,000
11.	None	11.	0.1 M Sodium citrate tribasic dihydrate pH 5.6	11.	0.5 M Ammonium phosphate monobasic
12.	0.2 M Magnesium chloride hexahydrate	12.	0.1 M HEPES sodium pH 7.5	12.	15% v/v 2-Propanol
13.	0.2 M Sodium citrate tribasic dihydrate	13.	0.1 M TRIS hydrochloride pH 8.5	13.	15% v/v Polyethylene glycol 400
14.	0.2 M Calcium chloride dihydrate	14.	0.1 M HEPES sodium pH 7.5	14.	14% v/v Polyethylene glycol 400
15.	0.2 M Ammonium sulfate	15.	0.1 M Sodium cacodylate trihydrate pH 6.5	15.	15% w/v Polyethylene glycol 8,000
16.	None	16.	0.1 M HEPES sodium pH 7.5	16.	0.75 M Lithium sulfate monohydrate
17.	0.2 M Lithium sulfate monohydrate	17.	0.1 M TRIS hydrochloride pH 8.5	17.	15% w/v Polyethylene glycol 4,000
18.	0.2 M Magnesium acetate tetrahydrate	18.	0.1 M Sodium cacodylate trihydrate pH 6.5	18.	10% w/v Polyethylene glycol 8,000
19.	0.2 M Ammonium acetate	19.	0.1 M TRIS hydrochloride pH 8.5	19.	15% v/v 2-Propanol
20.	0.2 M Ammonium sulfate	20.	0.1 M Sodium acetate trihydrate pH 4.6	20.	12.5% w/v Polyethylene glycol 4,000
21.	0.2 M Magnesium acetate tetrahydrate	21.	0.1 M Sodium cacodylate trihydrate pH 6.5	21.	15% v/v (+/-)-2-Methyl-2,4-pentanediol
22.	0.2 M Sodium acetate trihydrate	22.	0.1 M TRIS hydrochloride pH 8.5	22.	15% w/v Polyethylene glycol 4,000
23.	0.2 M Magnesium chloride hexahydrate	23.	0.1 M HEPES sodium pH 7.5	23.	15% v/v Polyethylene glycol 400
24.	0.2 M Calcium chloride dihydrate	24.	0.1 M Sodium acetate trihydrate pH 4.6	24.	10% v/v 2-Propanol
25.	None	25.	0.1 M Imidazole pH 6.5	25.	0.5 M Sodium acetate trihydrate
26.	0.2 M Ammonium acetate	26.	0.1 M Sodium citrate tribasic dihydrate pH 5.6	26.	15% v/v (+/-)-2-Methyl-2,4-pentanediol
27.	0.2 M Sodium citrate tribasic dihydrate	27.	0.1 M HEPES sodium pH 7.5	27.	10% v/v 2-Propanol
28.	0.2 M Sodium acetate trihydrate	28.	0.1 M Sodium cacodylate trihydrate pH 6.5	28.	15% w/v Polyethylene glycol 8,000
29.	None	29.	0.1 M HEPES sodium pH 7.5	29.	0.4 M Potassium sodium tartrate tetrahydrate
30.	0.2 M Ammonium sulfate	30.	None	30.	15% w/v Polyethylene glycol 8,000
31.	0.2 M Ammonium sulfate	31.	None	31.	15% w/v Polyethylene glycol 4,000
32.	None	32.	None	32.	1.0 M Ammonium sulfate
33.	None	33.	None	33.	2.0 M Sodium formate
34.	None	34.	0.1 M Sodium acetate trihydrate pH 4.6	34.	1.0 M Sodium formate
35.	None	35.	0.1 M HEPES sodium pH 7.5	35.	0.4 M Sodium phosphate monobasic monohydrate 0.4 M Potassium phosphate monobasic
36.	None	36.	0.1 M TRIS hydrochloride pH 8.5	36.	4% w/v Polyethylene glycol 8,000
37.	None	37.	0.1 M Sodium acetate trihydrate pH 4.6	37.	4% w/v Polyethylene glycol 4,000
38.	None	38.	0.1 M HEPES sodium pH 7.5	38.	0.7 M Sodium citrate tribasic dihydrate
39.	None	39.	0.1 M HEPES sodium pH 7.5	39.	2% v/v Polyethylene glycol 400 1.0 M Ammonium sulfate
40.	None	40.	0.1 M Sodium citrate tribasic dihydrate pH 5.6	40.	10% v/v 2-Propanol 10% w/v Polyethylene glycol 4,000
41.	None	41.	0.1 M HEPES sodium pH 7.5	41.	5% v/v 2-Propanol 10% w/v Polyethylene glycol 4,000
42.	0.05 M Potassium phosphate monobasic	42.	None	42.	10% w/v Polyethylene glycol 8,000
43.	None	43.	None	43.	15% w/v Polyethylene glycol 1,500
44.	None	44.	None	44.	0.1 M Magnesium formate dihydrate
45.	0.2 M Zinc acetate dihydrate	45.	0.1 M Sodium cacodylate trihydrate pH 6.5	45.	9% w/v Polyethylene glycol 8,000
46.	0.2 M Calcium acetate hydrate	46.	0.1 M Sodium cacodylate trihydrate pH 6.5	46.	9% w/v Polyethylene glycol 8,000
47.	None	47.	0.1 M Sodium acetate trihydrate pH 4.6	47.	1.0 M Ammonium sulfate
48.	None	48.	0.1 M TRIS hydrochloride pH 8.5	48.	1.0 M Ammonium phosphate monobasic
49.	0.5 M Lithium sulfate monohydrate	49.	None	49.	2% w/v Polyethylene glycol 8,000
50.	0.5 M Lithium sulfate monohydrate	50.	None	50.	7.5% w/v Polyethylene glycol 8,000

◇ Buffer pH is that of a 1.0 M stock prior to dilution with other reagent components: pH with HCl or NaOH.

Crystal Screen Lite contains fifty unique reagents. To determine the formulation of each reagent, simply read across the page.

CUSTOM SCREEN LITE (replaced the kit "Crystal screen cryo")

Tube	Component 1	Conc. 1	Unit 1	pH	Component 2	Conc. 2	Unit 2	Component 3	Conc. 3	Unit 3	pH	Component 4	Conc. 4	Unit 4	pH
1	PEG 6K	5	% w/v		sodium chloride	1	M	magnesium chloride hexahydrate	0.01	M					
2	hexadecyltrimethylammonium bromide	0.01	M		sodium chloride	0.25	M								
3	ethylene glycol	12.5	% v/v												
4	1,4 dioxane	18	% v/v												
5	2 propanol	5	% v/v		ammonium sulfate	1	M								
6	imidazole	0.5	M	7											
7	PEG 1K	5	% w/v					PEG 8K		5	% w/v				
8	ethanol	5	% v/v		sodium chloride	0.75	M								
9	sodium chloride	1	M					sodium acetate trihydrate	0.1	M	4.6				
10	MPD	15	% v/v		sodium chloride	0.2	M	sodium acetate trihydrate	0.1	M	4.6				
11	1,6 hexanediol	0.5	M		cobalt (II) chloride hexahydrate	0.01	M	sodium acetate trihydrate	0.1	M	4.6				
12	PEG 400	15	% v/v		cadmium chloride hydrate	0.1	M	sodium acetate trihydrate	0.1	M	4.6				
13	PEG 2K MME	15	% w/v		ammonium sulfate	0.2	M	sodium acetate trihydrate	0.1	M	4.6				
14	ammonium sulfate	1	M		sodium potassium tartrate tetrahydrate	0.2	M	sodium citrate tribasic dihydrate	0.1	M	5.6				
15	lithium sulfate monohydrate	0.5	M		ammonium sulfate	0.25	M	sodium citrate tribasic dihydrate	0.1	M	5.6				
16	ethylene imine polymer	2	% v/v		sodium chloride	0.25	M	sodium citrate tribasic dihydrate	0.1	M	5.6				
17	tert butanol	18	% v/v					sodium citrate tribasic dihydrate	0.1	M	5.6				
18	jeffamine M 600	5	% v/v		iron (III) chloride hexahydrate	0.01	M	sodium citrate tribasic dihydrate	0.1	M	5.6				
19	1,6 hexanediol	1.25	M					sodium citrate tribasic dihydrate	0.1	M	5.6				
20	magnesium sulfate heptahydrate	0.8	M					MES monohydrate	0.1	M	6.5				
21	sodium chloride	1	M		sodium phosphate monobasic monohydrate	0.1	M	potassium phosphate monobasic	0.1	M		MES monohydrate	0.1	M	6.5
22	PEG 20K	6	% w/v					MES monohydrate	0.1	M	6.5				
23	1,4 dioxane	5	% v/v		ammonium sulfate	0.8	M	MES monohydrate	0.1	M	6.5				
24	jeffamine M 600	15	% v/v		cesium chloride	0.05	M	MES monohydrate	0.1	M	6.5				
25	ammonium sulfate	0.9	M		cobalt (II) chloride hexahydrate	0.01	M	MES monohydrate	0.1	M	6.5				
26	PEG 5K MME	15	% w/v		ammonium sulfate	0.2	M	MES monohydrate	0.1	M	6.5				
27	PEG 550 MME	12.5	% v/v		zinc sulfate heptahydrate	0.01	M	MES monohydrate	0.1	M	6.5				
28	sodium citrate tribasic dihydrate	0.8	M	6.5											
29	MPD	15	% v/v		ammonium sulfate	0.5	M	HEPES	0.1	M	7.5				
30	PEG 6K	5	% w/v					MPD	2.5	% v/v		HEPES	0.1	M	7.5
31	jeffamine M 600	10	% v/v					HEPES	0.1	M	7.5				
32	ammonium sulfate	0.8	M		sodium chloride	0.1	M	HEPES	0.1	M	7.5				
33	ammonium formate	1	M					HEPES	0.1	M	7.5				
34	sodium acetate trihydrate	0.5	M		cadmium sulfate hydrate	0.05	M	HEPES	0.1	M	7.5				
35	MPD	35	% v/v					HEPES	0.1	M	7.5				
36	sodium chloride	2.2	M					HEPES	0.1	M	7.5				
37	PEG 8K	5	% w/v					ethylene glycol	4	% v/v		HEPES	0.1	M	7.5
38	PEG 10K	10	% w/v					HEPES	0.1	M	7.5				
39	1,6 hexanediol	1.7	M		magnesium chloride hexahydrate	0.2	M	tris	0.1	M	8.5				
40	tert butanol	13	% v/v					tris	0.1	M	8.5				
41	lithium sulfate monohydrate	0.5	M		nickel (II) chloride hexahydrate	0.01	M	tris	0.1	M	8.5				
42	glycerol	6	% v/v		ammonium sulfate	0.75	M	tris	0.1	M	8.5				
43	MPD	25	% v/v		ammonium phosphate monobasic	0.2	M	tris	0.1	M	8.5				
44	ethanol	10	% v/v					tris	0.1	M	8.5				
45	PEG 2K MME	10	% w/v		nickel (II) chloride hexahydrate	0.01	M	tris	0.1	M	8.5				
46	PEG 550 MME	10	% v/v		sodium chloride	0.1	M	bicine	0.1	M	9				
47	magnesium chloride hexahydrate	1	M					bicine	0.1	M	9				
48	1,4 dioxane	2	% v/v					PEG 20K	5	% w/v		bicine	0.1	M	9