

PEG/Ion 2 Screen™ is a crystallization reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules in the presence of Polyethylene glycol (3,350) and an array of neutralized and pH adjusted organic acids, multivalent ions, a novel Citrate BIS-TRIS propane buffer system and pH. PEG/Ion 2 Screen utilizes a monodisperse (Mr 3,300-3,400), high purity, Polyethylene glycol 3,350.

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, 3).

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 PEG/Ion 2 Screen 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.

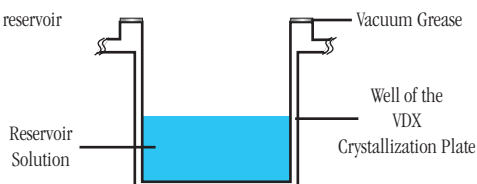
Performing The Screen

Since it is the most frequently reported method of crystallization, the following procedure describes the use of the PEG/Ion 2 Screen with the Hanging Drop Vapor Diffusion method. The PEG/Ion 2 Screen 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). Forty-eight reservoirs are to be prepared for a complete PEG/Ion 2 Screen. See Figure 1.

Figure 1

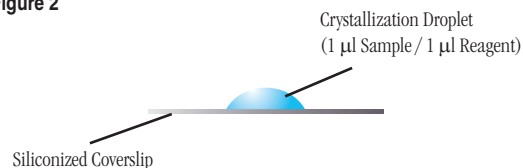
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of PEG/Ion 2 Screen reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of PEG/Ion 2 Screen reagent 2 into reservoir A2. Repeat the procedure for the remaining 46 PEG/Ion 2 Screen reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

3. Pipet 1 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

Figure 2

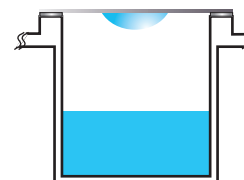


4. Pipet 1 µl of PEG/Ion 2 Screen reagent 1 from reservoir A1 into the sample droplet. See Figure 2.

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 47 PEG/Ion 2 Screen reagents.

7. If the quantity of sample permits, perform the PEG/Ion 2 Screen 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.

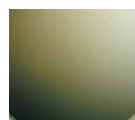
Examine The Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week thereafter. Records should indicate whether the drop is clear, contains precipitate, and/or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 4 (on page 2) shows typical examples of what one might observe in a crystallization experiment.

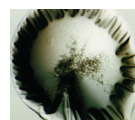
Interpreting PEG/Ion 2 Screen

Clear drops indicate that either the relative supersaturation of the sample and reagent is too low or the drop has not yet completed equilibration. If more than 33 of the 48 PEG/Ion 2 Screen drops are clear consider doubling the sample concentration and repeating the entire screen.

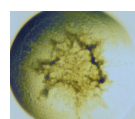
Figure 4
Typical observations in a crystallization experiment



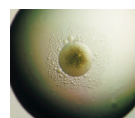
Clear Drop



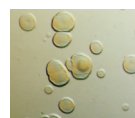
Skin/
Precipitate



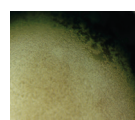
Precipitate



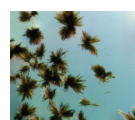
Precipitate/
Phase



Quasi
Crystals



Microcrystals



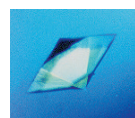
Needle
Cluster



Plates



Rod Cluster



Single
Crystal

Drops containing precipitate indicate that either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold and repeat the PEG/Ion 2 Screen condition. If more than 33 of the 48 PEG/Ion 2 Screen drops contain precipitate and no crystals are present, consider diluting the sample concentration in half and repeating the entire screen. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, glycerol, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue homogeneity. It is possible to obtain crystals from precipitate so do not discard nor ignore a drop containing precipitate. If possible, examine drops containing precipitate under polarizing optics to differentiate precipitate from microcrystalline material.

If the drop contains a macromolecular crystal the relative supersaturation of the sample and reagent is good. The next step is to optimize the preliminary conditions (pH, salt type, salt concentration, precipitant type, precipitant concentration, sample concentration, temperature, additives, and other crystallization variables) which produced the crystal in order to improve crystal size and quality.

Compare the observations between the 4°C and room temperature incubation to determine the effect of temperature on sample solubility. Different results in the same drops at different temperatures indicate that sample solubility is temperature dependent and that one should include temperature as a variable in subsequent screens and optimization experiments.

Retain and observe plates until the drops are dried out. Crystal growth can occur within 15 minutes or one year.

PEG/Ion 2 Screen Formulation

PEG/Ion 2 Screen reagents are formulated using the highest purity chemicals, ultrapure water (18.2 Megohm-cm, 5 ppb TOC) and are sterile filtered using 0.22 micron filters into sterile containers (no preservatives added).

PEG/Ion 2 reagents are readily reproduced using Hampton Research Optimize™ stock solutions of salts, polymers and buffers. Optimize stock reagents make reproducing PEG/Ion 2 Screen reagents fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize stock reagents.

For further details about formulation, reproducing and optimizing reagents from the PEG/Ion 2 Screen please refer to PEG/Ion 2 Screen Fundamentals.

PEG/Ion 2 Screen reagents are stable at room temperature and are best if used within 12 months of receipt. To enhance reagent stability it is recommended that PEG/Ion 2 Screen be stored at 4°C or -20°C. Avoid ultraviolet light to preserve reagent stability.

If the sample contains phosphate, borate, or carbonate buffers it is possible to obtain inorganic crystals (false positives) when using PEG/Ion 2 Screen reagents containing divalent cations such as magnesium, calcium, or zinc. To avoid false positives use phosphate, borate, or carbonate buffers at concentrations of 10 mM or less or exchange the phosphate, borate, or carbonate buffer with a more soluble buffer that does not complex with divalent cations.

References and Readings

1. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giegé, The Practical Approach Series, Oxford Univ. Press, 1992.
2. Current approaches to macromolecular crystallization. McPherson, A. Eur. J. Biochem. 189, 1-23, 1990.
3. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990.

Technical Support

Inquiries regarding PEG/Ion 2 Screen reagent formulation, 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 4:30 p.m. USA Pacific Standard Time.

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How to Reproduce PEG/Ion 2 Screen Reagents

PEG/Ion 2 Screen reagents and optimization conditions based on PEG/Ion 2 Screen 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 PEG/Ion 2 Screen reagent 1 in a crystallization plate.

Solution Composition: 0.1 M Sodium malonate pH 4.0
12% w/v Polyethylene glycol 3,350

- 731 μl water³
- 29 μl 3.4 M Sodium malonate pH 4.0
(CAS # 141-82-2, Catalog # HR2-747)
- 240 μl 50% w/v Polyethylene glycol 3,350
(CAS # 25322-68-3, Catalog # HR2-527)

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

Example 2. To prepare 10 milliliters of PEG/Ion 2 Screen reagent 37.

Solution Composition: 0.06 M Citric acid, 0.04 M BIS-TRIS propane/pH 4.1
16% w/v Polyethylene glycol 3,350

- 580 μl water³
- 40 μl 1.0 M BIS-TRIS propane
(CAS # 64431-96-5, Catalog # HR2-833)
- 60 μl 1.0 M Citric acid
(CAS # 77-92-9, Catalog # HR2-831)
- 320 μl 50% w/v Polyethylene glycol 3,350
(CAS # 25322-68-3, Catalog # HR2-527)

Make no pH adjustments. Mix well.

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

Formulation Notes for PEG/Ion 2 Screen Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the salts and buffers in Table 1 to reproduce a PEG/Ion 2 Screen 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.
7. Reagents 1-30 are formulated using titrated organic salts and Polyethylene glycol (Table 1). These reagents do not contain an additional buffer component. The indicated pH is that of the organic salt stock prior to dilution with Polyethylene glycol and water.
8. Reagents 31-35 are formulated from 1.0 M buffer stocks at the indicated pH, diluted to 0.15 M. These reagents also contain a specially formulated Tacsimate (see Salts Table 1). The buffer Tacsimate, and Polyethylene glycol are formulated together with no additional pH adjustment.
9. Reagents 36-41 are buffered using a novel Citrate BIS-TRIS propane (CBTP) system. By mixing different ratios of 1.0 M Citric acid and 1.0 M BIS-TRIS propane the CBTP reagent system can buffer between pH 3 and 9. A pH titration table for the CBTP buffer system is available at www.hamptonresearch.com by searching either catalog number HR2-831 or HR2-833.
10. Reagents 42, 44, 45, and 46 are formulated without a buffer.
11. The measured final pH of all PEG/Ion 2 reagents is available at www.hamptonresearch.com. Search using catalog number HR2-098 and follow the link to the 'PEG/Ion 2 pH and Conductivity' document.

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 PEG/Ion 2 Screen kit.

Optimize™ buffer stocks are supplied as a 100 milliliters sterile filtered solution.

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.

PEG/Ion 2 Screen™

HAMPTON
RESEARCH

Solutions for Crystal Growth

PEG/Ion 2 Screen Fundamentals

HR2-098 (pg 2)

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.

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 PEG/Ion 2 Screen and optimization reagents.

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 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 citrate tribasic pH 7.0	HR2-759	2.5 M	200 ml	3458-72-8
Ammonium tartrate dibasic pH 7.0	HR2-767	1.6 M	200 ml	3164-29-2
Calcium chloride dihydrate	HR2-557	2.0 M	100 ml	10035-04-8
Cadmium chloride hydrate	HR2-715	1.0 M	100 ml	654054-66-7
Cesium chloride	HR2-719	1.0 M	100 ml	7647-17-8
Cobalt(II) chloride hexahydrate	HR2-713	1.0 M	100 ml	7791-13-1
Magnesium chloride hexahydrate	HR2-559	2.0 M	100 ml	7791-18-6
	HR2-803	5.0 M	200 ml	7791-18-6
DL-Malic acid pH 7.0	HR2-761	3.0 M	200 ml	6915-15-7
Nickel(II) chloride hexahydrate	HR2-687	4.0 M	100 ml	7791-20-0
Sodium acetate trihydrate pH 7.0	HR2-763	4.0 M	200 ml	6131-90-4
Sodium bromide	HR2-699	5.0 M	200 ml	7647-15-6
Sodium formate pH 7.0	HR2-765	5.0 M	200 ml	141-53-7
Sodium malonate pH 4.0	HR2-747	3.4 M	200 ml	141-82-2
Sodium malonate pH 5.0	HR2-749	3.4 M	200 ml	141-82-2
Sodium malonate pH 6.0	HR2-751	3.4 M	200 ml	141-82-2
Sodium malonate pH 7.0	HR2-707	3.4 M	200 ml	141-82-2
Succinic acid pH 7.0	HR2-709	1.2 M	200 ml	110-15-6

(Continued on page 3)

PEG/Ion 2 Screen™

PEG/Ion 2 Screen Fundamentals

HR2-098 (pg 3)

Table 1 (Continued). Recommended reagents for the formulation of PEG/Ion 2 Screen and optimization reagents.

Salts	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Tacsimate™ pH 4.0	HR2-823	100%	200 ml	N/A
Tacsimate™ pH 5.0	HR2-825	100%	200 ml	N/A
Tacsimate™ pH 6.0	HR2-827	100%	200 ml	N/A
Tacsimate™ pH 7.0	HR2-755	100%	200 ml	N/A
Tacsimate™ pH 8.0	HR2-829	100%	200 ml	N/A
Tryptone	HR2-835	10% w/v	100 ml	91079-40-2
Zinc chloride	HR2-811	2.0 M	100 ml	7646-85-7
Polymer	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Polyethylene glycol 3,350	HR2-527	50% w/v	200 ml	25322-68-3
Buffers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
BIS-TRIS pH 6.5 ¹	HR2-783	1.0 M	100 ml	6976-37-0
BIS-TRIS propane	HR2-833	1.0 M	100 ml	64431-96-5
Citric acid	HR2-831	1.0 M	100 ml	77-92-9
HEPES pH 7.5 ²	HR2-729	1.0 M	100 ml	7365-45-9
HEPES sodium pH 7.0 ¹	HR2-931-03	1.0 M	185 ml	75277-39-3
Sodium acetate trihydrate pH 4.6 ¹	HR2-731	1.0 M	100 ml	6131-90-4
Sodium citrate tribasic dihydrate pH 5.6 ¹	HR2-735	1.0 M	100 ml	6132-04-3
Tris pH 8.5 ¹	HR2-725	1.0 M	100 ml	77-86-1
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 PEG/Ion 2 Screen and optimization reagents.

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
StockOptions™ Bis-Tris kit ⁴	HR2-106	1.0 M	10 ml each	6976-37-0	5.5 - 7.5
StockOptions™ Bis-Tris propane ⁴	HR2-103	1.0 M	10 ml each	64431-96-5	6.3 - 9.5

(Continued on page 4)

PEG/Ion 2 Screen™

Table 2 (Continued). **Recommended buffers for screening the pH of PEG/Ion 2 Screen and optimization reagents.**

Buffer Solution <u>or</u> Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
StockOptions™ Citric acid kit ⁴	HR2-104	1.0 M	10 ml each	77-92-9	2.2 - 6.5
StockOptions™ Hepes kit ⁴	HR2-102	1.0 M	10 ml each	7365-45-9	6.8 - 8.2
StockOptions™ Sodium Hepes kit ⁴	HR2-231	1.0 M	10 ml each	75277-39-9	6.8 - 8.2
StockOptions™ Sodium Acetate kit ⁴	HR2-233	1.0 M	10 ml each	6131-90-4	3.6 - 5.6
StockOptions™ Sodium Citrate kit ⁴	HR2-235	1.0 M	10 ml each	6132-04-3	4.2 - 6.5
StockOptions™ Tris kit ⁴	HR2-100	1.0 M	10 ml each	77-86-1	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 PEG/Ion 2 Screen 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 4:30 p.m. USA Pacific Standard Time.

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Tube #	Salt	Tube #	Buffer \diamond	Tube #	Polymer
1.	0.1 M Sodium malonate pH 4.0	1.	None	1.	12% w/v Polyethylene glycol 3,350
2.	0.2 M Sodium malonate pH 4.0	2.	None	2.	20% w/v Polyethylene glycol 3,350
3.	0.1 M Sodium malonate pH 5.0	3.	None	3.	12% w/v Polyethylene glycol 3,350
4.	0.2 M Sodium malonate pH 5.0	4.	None	4.	20% w/v Polyethylene glycol 3,350
5.	0.1 M Sodium malonate pH 6.0	5.	None	5.	12% w/v Polyethylene glycol 3,350
6.	0.2 M Sodium malonate pH 6.0	6.	None	6.	20% w/v Polyethylene glycol 3,350
7.	0.1 M Sodium malonate pH 7.0	7.	None	7.	12% w/v Polyethylene glycol 3,350
8.	0.2 M Sodium malonate pH 7.0	8.	None	8.	20% w/v Polyethylene glycol 3,350
9.	4% v/v Tacsimate™ pH 4.0	9.	None	9.	12% w/v Polyethylene glycol 3,350
10.	8% v/v Tacsimate™ pH 4.0	10.	None	10.	20% w/v Polyethylene glycol 3,350
11.	4% v/v Tacsimate™ pH 5.0	11.	None	11.	12% w/v Polyethylene glycol 3,350
12.	8% v/v Tacsimate™ pH 5.0	12.	None	12.	20% w/v Polyethylene glycol 3,350
13.	4% v/v Tacsimate™ pH 6.0	13.	None	13.	12% w/v Polyethylene glycol 3,350
14.	8% v/v Tacsimate™ pH 6.0	14.	None	14.	20% w/v Polyethylene glycol 3,350
15.	4% v/v Tacsimate™ pH 7.0	15.	None	15.	12% w/v Polyethylene glycol 3,350
16.	8% v/v Tacsimate™ pH 7.0	16.	None	16.	20% w/v Polyethylene glycol 3,350
17.	4% v/v Tacsimate™ pH 8.0	17.	None	17.	12% w/v Polyethylene glycol 3,350
18.	8% v/v Tacsimate™ pH 8.0	18.	None	18.	20% w/v Polyethylene glycol 3,350
19.	0.1 M Succinic acid pH 7.0	19.	None	19.	12% w/v Polyethylene glycol 3,350
20.	0.2 M Succinic acid pH 7.0	20.	None	20.	20% w/v Polyethylene glycol 3,350
21.	0.1 M Ammonium citrate tribasic pH 7.0	21.	None	21.	12% w/v Polyethylene glycol 3,350
22.	0.2 M Ammonium citrate tribasic pH 7.0	22.	None	22.	20% w/v Polyethylene glycol 3,350
23.	0.1 M DL-Malic acid pH 7.0	23.	None	23.	12% w/v Polyethylene glycol 3,350
24.	0.2 M DL-Malic acid pH 7.0	24.	None	24.	20% w/v Polyethylene glycol 3,350
25.	0.1 M Sodium acetate trihydrate pH 7.0	25.	None	25.	12% w/v Polyethylene glycol 3,350
26.	0.2 M Sodium acetate trihydrate pH 7.0	26.	None	26.	20% w/v Polyethylene glycol 3,350
27.	0.1 M Sodium formate pH 7.0	27.	None	27.	12% w/v Polyethylene glycol 3,350
28.	0.2 M Sodium formate pH 7.0	28.	None	28.	20% w/v Polyethylene glycol 3,350
29.	0.1 M Ammonium tartrate dibasic pH 7.0	29.	None	29.	12% w/v Polyethylene glycol 3,350
30.	0.2 M Ammonium tartrate dibasic pH 7.0	30.	None	30.	20% w/v Polyethylene glycol 3,350
31.	2% v/v Tacsimate™ pH 4.0	31.	0.1 M Sodium acetate trihydrate pH 4.6	31.	16% w/v Polyethylene glycol 3,350
32.	2% v/v Tacsimate™ pH 5.0	32.	0.1 M Sodium citrate tribasic dihydrate pH 5.6	32.	16% w/v Polyethylene glycol 3,350
33.	2% v/v Tacsimate™ pH 6.0	33.	0.1 M BIS-TRIS pH 6.5	33.	20% w/v Polyethylene glycol 3,350
34.	2% v/v Tacsimate™ pH 7.0	34.	0.1 M HEPES pH 7.5	34.	20% w/v Polyethylene glycol 3,350
35.	2% v/v Tacsimate™ pH 8.0	35.	0.1 M Tris pH 8.5	35.	16% w/v Polyethylene glycol 3,350
36.	None	36.	0.07 M Citric acid, 0.03 M BIS-TRIS propane / pH 3.4	36.	16% w/v Polyethylene glycol 3,350
37.	None	37.	0.06 M Citric acid, 0.04 M BIS-TRIS propane / pH 4.1	37.	16% w/v Polyethylene glycol 3,350
38.	None	38.	0.05 M Citric acid, 0.05 M BIS-TRIS propane / pH 5.0	38.	16% w/v Polyethylene glycol 3,350
39.	None	39.	0.04 M Citric acid, 0.06 M BIS-TRIS propane / pH 6.4	39.	20% w/v Polyethylene glycol 3,350
40.	None	40.	0.03 M Citric acid, 0.07 M BIS-TRIS propane / pH 7.6	40.	20% w/v Polyethylene glycol 3,350
41.	None	41.	0.02 M Citric acid, 0.08 M BIS-TRIS propane / pH 8.8	41.	16% w/v Polyethylene glycol 3,350
42.	0.02 M Calcium chloride dihydrate, 0.02 M Cadmium chloride hydrate, 0.02 M Cobalt(II) chloride hexahydrate	42.	None	42.	20% w/v Polyethylene glycol 3,350
43.	0.01 M Magnesium chloride hexahydrate 0.005 M Nickel(II) chloride hexahydrate	43.	0.1 M HEPES sodium pH 7.0	43.	15% w/v Polyethylene glycol 3,350
44.	0.02 M Zinc chloride	44.	None	44.	20% w/v Polyethylene glycol 3,350
45.	0.15 M Cesium chloride	45.	None	45.	15% w/v Polyethylene glycol 3,350
46.	0.2 M Sodium bromide	46.	None	46.	20% w/v Polyethylene glycol 3,350
47.	1% w/v Tryptone, 0.001 M Sodium azide	47.	0.05 M HEPES sodium pH 7.0	47.	12% w/v Polyethylene glycol 3,350
48.	1% w/v Tryptone, 0.001 M Sodium azide	48.	0.05 M HEPES sodium pH 7.0	48.	20% w/v Polyethylene glycol 3,350

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

PEG/Ion 2 Screen contains forty-eight unique reagents. To determine the formulation of each reagent, simply read across the page.

Sample: _____ Sample Concentration: _____
 Sample Buffer: _____ Date: _____
 Reservoir Volume: _____ Temperature: _____
 Drop Volume: Total _____ μ l Sample _____ μ l Reservoir _____ μ l Additive _____ μ l

- 1 Clear Drop
- 2 Phase Separation
- 3 Regular Granular Precipitate
- 4 Birefringent Precipitate or Microcrystals
- 5 Posettes or Spherulites
- 6 Needles (1D Growth)
- 7 Plates (2D Growth)
- 8 Single Crystals (3D Growth < 0.2 mm)
- 9 Single Crystals (3D Growth > 0.2 mm)

PEG/Ion 2 Screen™ - HR2-098 Scoring Sheet

Date: Date: Date: Date:

1.	0.1 M Sodium malonate pH 4.0, 12% w/v Polyethylene glycol 3,350				
2.	0.2 M Sodium malonate pH 4.0, 20% w/v Polyethylene glycol 3,350				
3.	0.1 M Sodium malonate pH 5.0, 12% w/v Polyethylene glycol 3,350				
4.	0.2 M Sodium malonate pH 5.0, 20% w/v Polyethylene glycol 3,350				
5.	0.1 M Sodium malonate pH 6.0, 12% w/v Polyethylene glycol 3,350				
6.	0.2 M Sodium malonate pH 6.0, 20% w/v Polyethylene glycol 3,350				
7.	0.1 M Sodium malonate pH 7.0, 12% w/v Polyethylene glycol 3,350				
8.	0.2 M Sodium malonate pH 7.0, 20% w/v Polyethylene glycol 3,350				
9.	4% v/v Tacsimate™ pH 4.0, 12% w/v Polyethylene glycol 3,350				
10.	8% v/v Tacsimate™ pH 4.0, 20% w/v Polyethylene glycol 3,350				
11.	4% v/v Tacsimate™ pH 5.0, 12% w/v Polyethylene glycol 3,350				
12.	8% v/v Tacsimate™ pH 5.0, 20% w/v Polyethylene glycol 3,350				
13.	4% v/v Tacsimate™ pH 6.0, 12% w/v Polyethylene glycol 3,350				
14.	8% v/v Tacsimate™ pH 6.0, 20% w/v Polyethylene glycol 3,350				
15.	4% v/v Tacsimate™ pH 7.0, 12% w/v Polyethylene glycol 3,350				
16.	8% v/v Tacsimate™ pH 7.0, 20% w/v Polyethylene glycol 3,350				
17.	4% v/v Tacsimate™ pH 8.0, 12% w/v Polyethylene glycol 3,350				
18.	8% v/v Tacsimate™ pH 8.0, 20% w/v Polyethylene glycol 3,350				
19.	0.1 M Succinic acid pH 7.0, 12% w/v Polyethylene glycol 3,350				
20.	0.2 M Succinic acid pH 7.0, 20% w/v Polyethylene glycol 3,350				
21.	0.1 M Ammonium citrate tribasic pH 7.0, 12% w/v Polyethylene glycol 3,350				
22.	0.2 M Ammonium citrate tribasic pH 7.0, 20% w/v Polyethylene glycol 3,350				
23.	0.1 M DL-Malic acid pH 7.0, 12% w/v Polyethylene glycol 3,350				
24.	0.2 M DL-Malic acid pH 7.0, 20% w/v Polyethylene glycol 3,350				
25.	0.1 M Sodium acetate trihydrate pH 7.0, 12% w/v Polyethylene glycol 3,350				
26.	0.2 M Sodium acetate trihydrate pH 7.0, 20% w/v Polyethylene glycol 3,350				
27.	0.1 M Sodium formate pH 7.0, 12% w/v Polyethylene glycol 3,350				
28.	0.2 M Sodium formate pH 7.0, 20% w/v Polyethylene glycol 3,350				
29.	0.1 M Ammonium tartrate dibasic pH 7.0, 12% w/v Polyethylene glycol 3,350				
30.	0.2 M Ammonium tartrate dibasic pH 7.0, 20% w/v Polyethylene glycol 3,350				
31.	2% v/v Tacsimate™ pH 4.0, 0.1 M Sodium acetate trihydrate pH 4.6, 16% w/v Polyethylene glycol 3,350				
32.	2% v/v Tacsimate™ pH 5.0, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 16% w/v Polyethylene glycol 3,350				
33.	2% v/v Tacsimate™ pH 6.0, 0.1 M BIS-TRIS pH 6.5, 20% w/v Polyethylene glycol 3,350				
34.	2% v/v Tacsimate™ pH 7.0, 0.1 M HEPES pH 7.5, 20% w/v Polyethylene glycol 3,350				
35.	2% v/v Tacsimate™ pH 8.0, 0.1 M Tris pH 8.5, 16% w/v Polyethylene glycol 3,350				
36.	(0.07 M Citric acid, 0.03 M BIS-TRIS propane / pH 3.4), 16% w/v Polyethylene glycol 3,350				
37.	(0.06 M Citric acid, 0.04 M BIS-TRIS propane / pH 4.1), 16% w/v Polyethylene glycol 3,350				
38.	(0.05 M Citric acid, 0.05 M BIS-TRIS propane / pH 5.0), 16% w/v Polyethylene glycol 3,350				
39.	(0.04 M Citric acid, 0.06 M BIS-TRIS propane / pH 6.4), 20% w/v Polyethylene glycol 3,350				
40.	(0.03 M Citric acid, 0.07 M BIS-TRIS propane / pH 7.6), 20% w/v Polyethylene glycol 3,350				
41.	(0.02 M Citric acid, 0.08 M BIS-TRIS propane / pH 8.8), 16% w/v Polyethylene glycol 3,350				
42.	0.02 M Calcium chloride dihydrate, 0.02 M Cadmium chloride hydrate, 0.02 M Cobalt(II) chloride hexahydrate, 20% w/v Polyethylene glycol 3,350				
43.	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				
44.	0.02 M Zinc chloride, 20% w/v Polyethylene glycol 3,350				
45.	0.15 M Cesium chloride, 15% w/v Polyethylene glycol 3,350				
46.	0.2 M Sodium bromide, 20% w/v Polyethylene glycol 3,350				
47.	1% w/v Tryptone, 0.001 M Sodium azide, 0.05 M HEPES sodium pH 7.0, 12% w/v Polyethylene glycol 3,350				
48.	1% w/v Tryptone, 0.001 M Sodium azide, 0.05 M HEPES sodium pH 7.0, 20% w/v Polyethylene glycol 3,350				



Solutions for Crystal Growth

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34 Journey

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