# PEG/Ion 2 Screen™

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*Solutions for Crystal Growth*

**User Guide** **HR2-098 (pg 1)**

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).

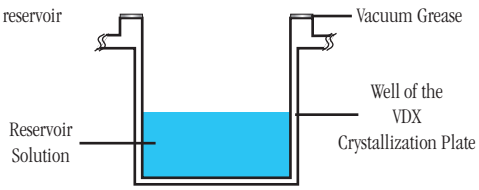
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the PEG/Ion 2 Screen variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against water although 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.

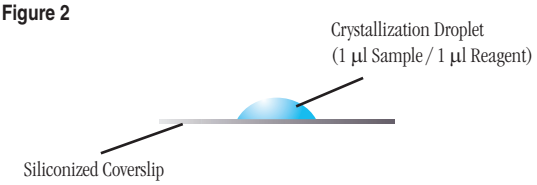
**Figure 1**
Cross section of a reservoir in the VDX plate.




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.

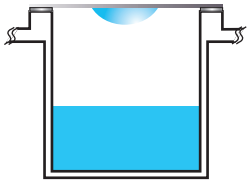
**Figure 2**




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 there after. 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.

# PEG/Ion 2 Screen™

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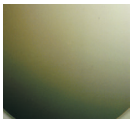
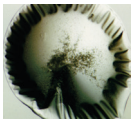
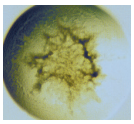
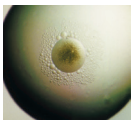
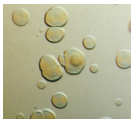
**Figure 4**
Typical observations in a crystallization experiment

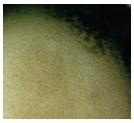

Clear Drop


Skin / Precipitate

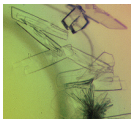

Precipitate


Precipitate / Phase

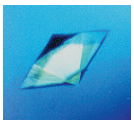

Quasi Crystals


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. Giege, 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.

Hampton Research
34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
Technical Support e-mail: tech@hrmail.com
Website: www.hamptonresearch.com

# PEG/Ion 2 Screen™

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## PEG/Ion 2 Screen Fundamentals HR2-098 (pg 1)

### 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 water[3]
- 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 water[3]
- 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.

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## 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™

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**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™

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**Table 2 (Continued). 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™ 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.

Hampton Research
34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
Technical Support e-mail: tech@hrmail.com
Website: www.hamptonresearch.com

| Tube # | Salt | Buffer ◊ | Polymer |
|---|---|---|---|
| 1. | 0.1 M Sodium malonate pH 4.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 2. | 0.2 M Sodium malonate pH 4.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 3. | 0.1 M Sodium malonate pH 5.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 4. | 0.2 M Sodium malonate pH 5.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 5. | 0.1 M Sodium malonate pH 6.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 6. | 0.2 M Sodium malonate pH 6.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 7. | 0.1 M Sodium malonate pH 7.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 8. | 0.2 M Sodium malonate pH 7.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 9. | 4% v/v Tacsimate™ pH 4.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 10. | 8% v/v Tacsimate™ pH 4.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 11. | 4% v/v Tacsimate™ pH 5.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 12. | 8% v/v Tacsimate™ pH 5.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 13. | 4% v/v Tacsimate™ pH 6.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 14. | 8% v/v Tacsimate™ pH 6.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 15. | 4% v/v Tacsimate™ pH 7.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 16. | 8% v/v Tacsimate™ pH 7.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 17. | 4% v/v Tacsimate™ pH 8.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 18. | 8% v/v Tacsimate™ pH 8.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 19. | 0.1 M Succinic acid pH 7.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 20. | 0.2 M Succinic acid pH 7.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 21. | 0.1 M Ammonium citrate tribasic pH 7.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 22. | 0.2 M Ammonium citrate tribasic pH 7.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 23. | 0.1 M DL-Malic acid pH 7.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 24. | 0.2 M DL-Malic acid pH 7.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 25. | 0.1 M Sodium acetate trihydrate pH 7.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 26. | 0.2 M Sodium acetate trihydrate pH 7.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 27. | 0.1 M Sodium formate pH 7.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 28. | 0.2 M Sodium formate pH 7.0 | None | 20% w/v Polyethylene glycol 3,350 |
| 29. | 0.1 M Ammonium tartrate dibasic pH 7.0 | None | 12% w/v Polyethylene glycol 3,350 |
| 30. | 0.2 M Ammonium tartrate dibasic pH 7.0 | None | 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. | None | 0.07 M Citric acid, 0.03 M BIS-TRIS propane / pH 3.4 | 16% w/v Polyethylene glycol 3,350 |
| 37. | None | 0.06 M Citric acid, 0.04 M BIS-TRIS propane / pH 4.1 | 16% w/v Polyethylene glycol 3,350 |
| 38. | None | 0.05 M Citric acid, 0.05 M BIS-TRIS propane / pH 5.0 | 16% w/v Polyethylene glycol 3,350 |
| 39. | None | 0.04 M Citric acid, 0.06 M BIS-TRIS propane / pH 6.4 | 20% w/v Polyethylene glycol 3,350 |
| 40. | None | 0.03 M Citric acid, 0.07 M BIS-TRIS propane / pH 7.6 | 20% w/v Polyethylene glycol 3,350 |
| 41. | None | 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 | None | 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 | None | 20% w/v Polyethylene glycol 3,350 |
| 45. | 0.15 M Cesium chloride | None | 15% w/v Polyethylene glycol 3,350 |
| 46. | 0.2 M Sodium bromide | None | 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 |

◊ 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.*

34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
E-mail: tech@hrmail.com
Website: www.hamptonresearch.com

HAMPTON RESEARCH
*Solutions for Crystal Growth*

Sample: _____ Sample Concentration: _____

Sample Buffer: _____ Date: _____

Reservoir Volume: _____ Temperature: _____

Drop Volume: Total _____ µl Sample _____ µl Reservoir _____ µl Additive _____ µl

**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)

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*Solutions for Crystal Growth*

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e-mail: tech@hrmail.com
Website: www.hamptonresearch.com



| 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 | | | | |