HAM #HR2126

PEG/Ion Screen™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-126**

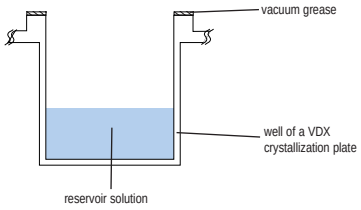
PEG/Ion Screen™ is a crystallization reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules in the presence of polyethylene glycol (3350) and 48 unique salts representing a very complete range of anions and cations frequently used in the crystallization of biological macromolecules. PEG/Ion Screen utilizes a monodisperse (Mr 3300-3400), high purity, polyethylene glycol 3350. The screen combines with this high purity PEG, 48 different high purity salts, comprising both anions (sulfate, nitrate, tartrate, acetate, chloride, iodide, thiocyanate, formate, citrate, phosphate, and fluoride) and cations (sodium, potassium, ammonium, lithium, magnesium, and calcium) in a relatively low concentration (0.2 M) which due to their unique pH characteristics also affords a reasonable pH screen (approximate pH range of 4 to 9). The primary screen variables are PEG, ion type, ionic strength, and pH. The screen is a straightforward, effective, and practical kit for determining preliminary crystallization conditions. Within the previous two years more than 60% of the published crystallization reports utilized polyethylene glycol as a primary crystallization reagent and in 50% of those reports the PEG was combined with a ion as a secondary crystallization reagent. PEG/Ion Screen is a crystallization screen matrix which is biased towards evaluating the most frequently reported crystallization combination within the previous two years - PEG and ionic strength. PEG/Ion Screen is also effective in determining the solubility of a macromolecule in a wide range of ions across a relatively broad pH range in the presence of polyethylene glycol.

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 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.

figure 1
Cross section of a reservoir in the VDX plate.



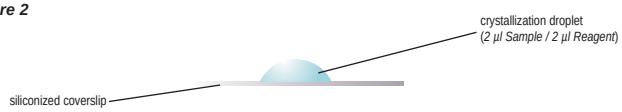
Performing The Screen

Since it is the most frequently reported method of crystallization, the following procedure describes the use of the PEG/Ion Screen with the Hanging Drop Vapor Diffusion method. The PEG/Ion 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 Screen. See figure 1.

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

figure 2

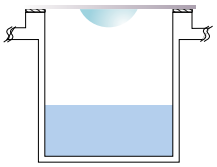


3. Pipet 2 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See figure 2.

4. Pipet 2 µl of PEG/Ion Screen 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 reservior.



6. Repeat operations 3 through 5 for the remaining 47 PEG/Ion Screen reagents.

7. If the quantity of sample permits, perform the PEG/Ion 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 the PEG/Ion 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 the drop remains clear after 3 to 4 weeks consider repeating the PEG/Ion Screen condition and doubling the sample concentration. If more than 33 of the 48 PEG/Ion Screen

PEG/Ion Screen™

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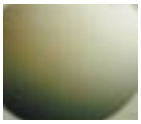
figure 4
Typical observations in a crystallization experiment.


Clear Drop


Skin/Precipitate


Precipitate


Precipitate/Phase


Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

drops are clear consider doubling the sample concentration and repeating the entire screen.

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 Screen condition. If more than 33 of the 48 PEG/Ion 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 Screen Formulation

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

No pH adjustments are made to PEG/Ion Screen. Reagent are combined without further titration.

PEG/Ion Screen reagents are stable at room temperature and are best used before the "Best If Used By" date on the kit tubes. To enhance reagent stability it is strongly recommended that PEG/Ion 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 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 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 5:00 p.m. USA Pacific Standard Time.

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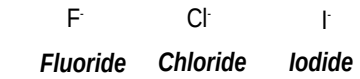
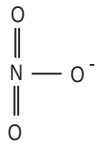
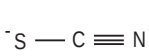
Tube Number	Salt	Polyethylene Glycol 3350	pH
1.	0.2 M Sodium Fluoride	20% w/v Polyethylene Glycol 3350	7.1
2.	0.2 M Potassium Fluoride	20% w/v Polyethylene Glycol 3350	7.2
3.	0.2 M Ammonium Fluoride	20% w/v Polyethylene Glycol 3350	6.2
4.	0.2 M Lithium Chloride anhydrous	20% w/v Polyethylene Glycol 3350	6.7
5.	0.2 M Magnesium Chloride hexahydrate	20% w/v Polyethylene Glycol 3350	5.8
6.	0.2 M Sodium Chloride	20% w/v Polyethylene Glycol 3350	6.9
7.	0.2 M Calicium Chloride dihydrate	20% w/v Polyethylene Glycol 3350	5.1
8.	0.2 M Potassium Chloride	20% w/v Polyethylene Glycol 3350	6.9
9.	0.2 M Ammonium Chloride	20% w/v Polyethylene Glycol 3350	6.3
10.	0.2 M Sodium Iodide	20% w/v Polyethylene Glycol 3350	6.9
11.	0.2 M Potassium Iodide	20% w/v Polyethylene Glycol 3350	6.8
12.	0.2 M Ammonium Iodide	20% w/v Polyethylene Glycol 3350	6.2
13.	0.2 M Sodium Thiocyanate	20% w/v Polyethylene Glycol 3350	6.9
14.	0.2 M Potassium Thiocyanate	20% w/v Polyethylene Glycol 3350	7.0
15.	0.2 M Lithium Nitrate	20% w/v Polyethylene Glycol 3350	7.1
16.	0.2 M Magnesium Nitrate hexahydrate	20% w/v Polyethylene Glycol 3350	5.8
17.	0.2 M Sodium Nitrate	20% w/v Polyethylene Glycol 3350	6.8
18.	0.2 M Potassium Nitrate	20% w/v Polyethylene Glycol 3350	6.9
19.	0.2 M Ammonium Nitrate	20% w/v Polyethylene Glycol 3350	6.3
20.	0.2 M Magnesium Formate	20% w/v Polyethylene Glycol 3350	5.9
21.	0.2 M Sodium Formate	20% w/v Polyethylene Glycol 3350	7.2
22.	0.2 M Potassium Formate	20% w/v Polyethylene Glycol 3350	7.3
23.	0.2 M Ammonium Formate	20% w/v Polyethylene Glycol 3350	6.6
24.	0.2 M Lithium Acetate dihydrate	20% w/v Polyethylene Glycol 3350	7.8
25.	0.2 M Magnesium Acetate tetrahydrate	20% w/v Polyethylene Glycol 3350	7.7
26.	0.2 M Zinc Acetate dihydrate	20% w/v Polyethylene Glycol 3350	6.3
27.	0.2 M Sodium Acetate trihydrate	20% w/v Polyethylene Glycol 3350	7.9
28.	0.2 M Calcium Acetate hydrate	20% w/v Polyethylene Glycol 3350	7.3
29.	0.2 M Potassium Acetate	20% w/v Polyethylene Glycol 3350	7.8
30.	0.2 M Ammonium Acetate	20% w/v Polyethylene Glycol 3350	7.1
31.	0.2 M Lithium Sulfate monohydrate	20% w/v Polyethylene Glycol 3350	6.4
32.	0.2 M Magnesium Sulfate heptahydrate	20% w/v Polyethylene Glycol 3350	5.9
33.	0.2 M Sodium Sulfate decahydrate	20% w/v Polyethylene Glycol 3350	6.6
34.	0.2 M Potassium Sulfate	20% w/v Polyethylene Glycol 3350	6.7
35.	0.2 M Ammonium Sulfate	20% w/v Polyethylene Glycol 3350	6.0
36.	0.2 M di-Sodium Tartrate dihydrate	20% w/v Polyethylene Glycol 3350	7.2
37.	0.2 M Potassium Sodium Tartrate tetrahydrate	20% w/v Polyethylene Glycol 3350	7.2
38.	0.2 M di-Ammonium Tartrate	20% w/v Polyethylene Glycol 3350	6.6
39.	0.2 M Sodium dihydrogen Phosphate monohydrate	20% w/v Polyethylene Glycol 3350	4.5
40.	0.2 M di-Sodium hydrogen Phosphate dihydrate	20% w/v Polyethylene Glycol 3350	9.1
41.	0.2 M Potassium dihydrogen Phosphate	20% w/v Polyethylene Glycol 3350	4.7
42.	0.2 M di-Potassium hydrogen Phosphate	20% w/v Polyethylene Glycol 3350	9.2
43.	0.2 M Ammonium dihydrogen Phosphate	20% w/v Polyethylene Glycol 3350	4.6
44.	0.2 M di-Ammonium hydrogen Phosphate	20% w/v Polyethylene Glycol 3350	7.9
45.	0.2 M tri-Lithium Citrate tetrahydrate	20% w/v Polyethylene Glycol 3350	8.1
46.	0.2 M tri-Sodium Citrate dihydrate	20% w/v Polyethylene Glycol 3350	8.2
47.	0.2 M tri-Potassium Citrate monohydrate	20% w/v Polyethylene Glycol 3350	8.3
48.	0.2 M di-Ammonium hydrogen Citrate	20% w/v Polyethylene Glycol 3350	5.0



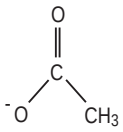
Fluoride ***Chloride*** ***Iodide***



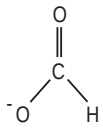
Nitrate



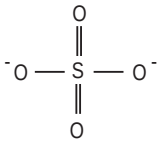
Thiocyanate



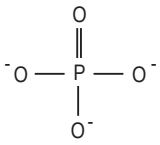
Acetate



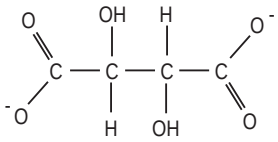
Formate



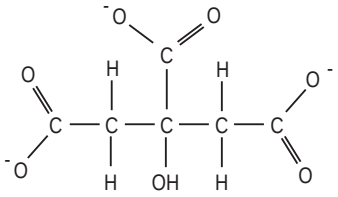
Sulfate



Phosphate



Tartrate



Citrate

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

34 Journey
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Sample: _____ *Sample Concentration:* _____

Sample Buffer: _____ *Date:* _____

Reservior Volume: _____ *Temperature:* _____

Drop Volume: Total _____ *µl Sample* _____ *µl Reservior* _____ *µl Additive* _____ *µl*

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

PEG/Ion - Scoring Sheet	Date:	Date:	Date:	Date:	Date:
1. 0.2 M Sodium Fluoride, 20% PEG 3350					
2. 0.2 M Potassium Fluoride, 20% PEG 3350					
3. 0.2 M Ammonium Fluoride, 20% PEG 3350					
4. 0.2 M Lithium Chloride, 20% PEG 3350					
5. 0.2 M Magnesium Chloride, 20% PEG 3350					
6. 0.2 M Sodium Chloride, 20% PEG 3350					
7. 0.2 M Calcium Chloride, 20% PEG 3350					
8. 0.2 M Potassium Chloride, 20% PEG 3350					
9. 0.2 M Ammonium Chloride, 20% PEG 3350					
10. 0.2 M Sodium Iodide, 20% PEG 3350					
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12. 0.2 M Ammonium Iodide, 20% PEG 3350					
13. 0.2 M Sodium Thiocyanate, 20% PEG 3350					
14. 0.2 M Potassium Thiocyanate, 20% PEG 3350					
15. 0.2 M Lithium Nitrate, 20% PEG 3350					
16. 0.2 M Magnesium Nitrate, 20% PEG 3350					
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37. 0.2 M Potassium Sodium Tartrate, 20% PEG 3350					
38. 0.2 M di-Ammonium Tartrate, 20% PEG 3350					
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41. 0.2 M Potassium dihydrogen Phosphate, 20% PEG 3350					
42. 0.2 M di-Potassium hydrogen Phosphate, 20% PEG 3350					
43. 0.2 M Ammonium dihydrogen Phosphate, 20% PEG 3350					
44. 0.2 M di-Ammonium hydrogen Phosphate, 20% PEG 3350					
45. 0.2 M tri-Lithium Citrate, 20% PEG 3350					
46. 0.2 M tri-Sodium Citrate, 20% PEG 3350					
47. 0.2 M tri-Potassium Citrate, 20% PEG 3350					
48. 0.2 M di-Ammonium hydrogen Citrate, 20% PEG 3350					

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