Grid Screen™

PEG 6000

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-213 (pg 1)**

Grid Screen™ PEG 6000 is a preformulated reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules. The screen is simple and practical for finding initial crystallization conditions as well as determining the solubility of a macromolecule in Polyethylene glycol 6,000 between pH 4.0 and 9.0.

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]

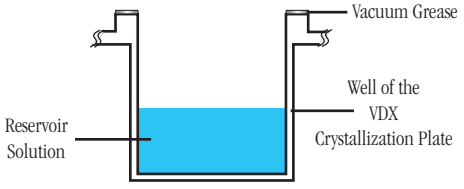
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Grid Screen PEG 6000 variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against water although 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 Grid Screen PEG 6000 with the Hanging Drop Vapor Diffusion method. Grid Screen PEG 6000 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 and HR3-142) 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 VDX™ Plate with sealant (HR3-170 and HR3-172). Twenty-four reservoirs are to be prepared for a complete Grid Screen PEG 6000. See Figure 1.

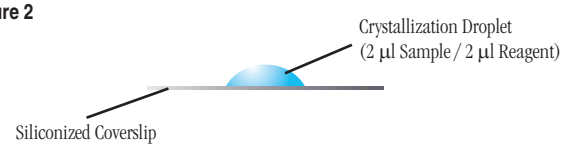
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Grid Screen PEG 6000 reagent A1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of Grid Screen PEG 6000 reagent A2 into reservoir A2. Repeat the procedure for the remaining 22 Grid Screen PEG 6000 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.

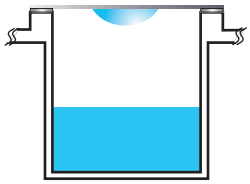
Figure 2



4. Pipet 2 µl of Grid Screen PEG 6000 reagent A1 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 23 Grid Screen PEG 6000 reagents.

7. If the quantity of sample permits, perform Grid Screen PEG 6000 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 Grid Screen PEG 6000

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 Grid Screen PEG 6000 condition and doubling the sample concentration. If more than 70%

Grid Screen™

PEG 6000

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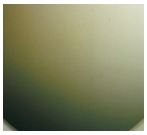
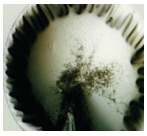
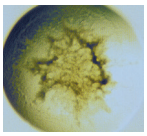
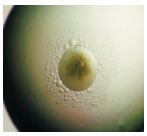
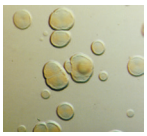
Figure 4
Typical observations in a crystallization experiment

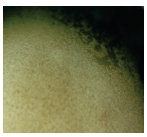

Clear Drop


Skin/Precipitate

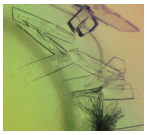

Precipitate


Precipitate/Phase

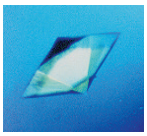

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Grid Screen PEG 6000 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 Grid Screen PEG 6000 condition. If more than 70% Grid Screen PEG 6000 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.

Grid Screen PEG 6000 Formulation

Grid Screen PEG 6000 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).

Grid Screen PEG 6000 reagents are stable at room temperature and are best if used within 12 months of receipt. To enhance reagent stability it is strongly recommended that Grid Screen PEG 6000 be stored at 4°C or -20°C. Avoid ultraviolet light to preserve reagent stability.

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.
4. Advance in Protein Chemistry Volume 41. Pages 1-33 (Patricia C. Weber). Academic Press, 1991.
5. Current approaches to macromolecular crystallization., McPherson, A., Eur. J. Biochem. 189, 1-23, 1990.
6. Crystallization of Membrane Proteins. Edited by Hartmut Michel,1990. CRC Press.

Technical Support

Inquiries regarding Grid Screen PEG 6000 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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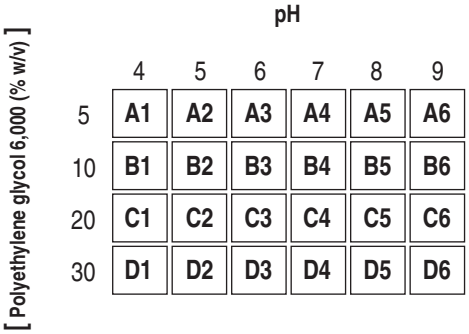

The pH indicated on each Grid Screen reagent is the ACTUAL pH of the reagent at 25.0 ° C. All pH adjustments have been made using Hydrochloric acid or Sodium hydroxide.

Tube #	Polyethylene glycol 6,000 [% w/v]	Tube #	Buffer
A1.	5	A1.	0.1 M Citric acid pH 4.0
B1.	10	B1.	0.1 M Citric acid pH 4.0
C1.	20	C1.	0.1 M Citric acid pH 4.0
D1.	30	D1.	0.1 M Citric acid pH 4.0
A2.	5	A2.	0.1 M Citric acid pH 5.0
B2.	10	B2.	0.1 M Citric acid pH 5.0
C2.	20	C2.	0.1 M Citric acid pH 5.0
D2.	30	D2.	0.1 M Citric acid pH 5.0
A3.	5	A3.	0.1 M MES monohydrate pH 6.0
B3.	10	B3.	0.1 M MES monohydrate pH 6.0
C3.	20	C3.	0.1 M MES monohydrate pH 6.0
D3.	30	D3.	0.1 M MES monohydrate pH 6.0
A4.	5	A4.	0.1 M HEPES pH 7.0
B4.	10	B4.	0.1 M HEPES pH 7.0
C4.	20	C4.	0.1 M HEPES pH 7.0
D4.	30	D4.	0.1 M HEPES pH 7.0
A5.	5	A5.	0.1 M Tris pH 8.0
B5.	10	B5.	0.1 M Tris pH 8.0
C5.	20	C5.	0.1 M Tris pH 8.0
D5.	30	D5.	0.1 M Tris pH 8.0
A6.	5	A6.	0.1 M BICINE pH 9.0
B6.	10	B6.	0.1 M BICINE pH 9.0
C6.	20	C6.	0.1 M BICINE pH 9.0
D6.	30	D6.	0.1 M BICINE pH 9.0

Chemical Analysis and Recommended Optimization Reagents

HR2-533 - 50% w/v Polyethylene glycol 6,000, 200 milliliters	M_r 5,000-7,000	$H(OCH_2CH_2)_nOH$	CAS Number [25322-68-3]	EC No 500-038-2
HR2-831 - 1.0 M Citric acid, 100 milliliters	M_r 192.13	$C_6H_8O_7$	CAS Number [77-92-9]	EC No 201-069-1
HR2-587 - 0.5 M MES monohydrate, 100 milliliters	M_r 213.25	$C_6H_{13}NO_4S \cdot H_2O$	CAS Number [145224-94-8]	EC No 224-632-3
HR2-585 - 1.0 M HEPES, 100 milliliters	M_r 238.31	$C_8H_{18}N_2O_4S$	CAS Number [7365-45-9]	EC No 230-907-9
HR2-589 - 1.0 M Tris, 100 milliliters	M_r 121.14	$C_4H_{11}NO_3$	CAS Number [77-86-1]	EC No 201-064-4
HR2-509 - 1.0 M BICINE, 100 milliliters	M_r 163.17	$C_6H_{13}NO_4$	CAS Number [150-25-4]	EC No 205-755-1

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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)

Grid Screen™ PEG 6000 - HR2-213 Scoring Sheet	Date:	Date:	Date:	Date:
A1. 0.1 M Citric acid pH 4.0, 5% w/v Polyethylene glycol 6,000				
A2. 0.1 M Citric acid pH 5.0, 5% w/v Polyethylene glycol 6,000				
A3. 0.1 M MES monohydrate pH 6.0, 5% w/v Polyethylene glycol 6,000				
A4. 0.1 M HEPES pH 7.0, 5% w/v Polyethylene glycol 6,000				
A5. 0.1 M Tris pH 8.0, 5% w/v Polyethylene glycol 6,000				
A6. 0.1 M BICINE pH 9.0, 5% w/v Polyethylene glycol 6,000				
B1. 0.1 M Citric acid pH 4.0, 10% w/v Polyethylene glycol 6,000				
B2. 0.1 M Citric acid pH 5.0, 10% w/v Polyethylene glycol 6,000				
B3. 0.1 M MES monohydrate pH 6.0, 10% w/v Polyethylene glycol 6,000				
B4. 0.1 M HEPES pH 7.0, 10% w/v Polyethylene glycol 6,000				
B5. 0.1 M Tris pH 8.0, 10% w/v Polyethylene glycol 6,000				
B6. 0.1 M BICINE pH 9.0, 10% w/v Polyethylene glycol 6,000				
C1. 0.1 M Citric acid pH 4.0, 20% w/v Polyethylene glycol 6,000				
C2. 0.1 M Citric acid pH 5.0, 20% w/v Polyethylene glycol 6,000				
C3. 0.1 M MES monohydrate pH 6.0, 20% w/v Polyethylene glycol 6,000				
C4. 0.1 M HEPES pH 7.0, 20% w/v Polyethylene glycol 6,000				
C5. 0.1 M Tris pH 8.0, 20% w/v Polyethylene glycol 6,000				
C6. 0.1 M BICINE pH 9.0, 20% w/v Polyethylene glycol 6,000				
D1. 0.1 M Citric acid pH 4.0, 30% w/v Polyethylene glycol 6,000				
D2. 0.1 M Citric acid pH 5.0, 30% w/v Polyethylene glycol 6,000				
D3. 0.1 M MES monohydrate pH 6.0, 30% w/v Polyethylene glycol 6,000				
D4. 0.1 M HEPES pH 7.0, 30% w/v Polyethylene glycol 6,000				
D5. 0.1 M Tris pH 8.0, 30% w/v Polyethylene glycol 6,000				
D6. 0.1 M BICINE pH 9.0, 30% w/v Polyethylene glycol 6,000				

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