PEGRx™ 1

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-082 (pg 1)**

Description

PEGRx™ 1 is a crystallization reagent kit designed to evaluate an array of polymers of varying molecular weight in a low ionic strength environment versus a wide range of pH. Polymer reagents include Polyethylene glycols, Polyethylene glycol monomethyl ethers, and Jeffamines®. The molecular weight range between 200 and 20,000 is evaluated in a low ionic strength formulation. Ten different buffers are used to span the range of pH between 3.5 and 9. The primary screen variables are polymer type, polymer molecular weight, pH and low ionic strength. PEGRx 1 is a straightforward, effective, and efficient screen for determining preliminary crystallization conditions in a low ionic strength, polymeric reagent formulation in the pH range 3.5 to 9. The choice of polymers, polymer concentration, and pH were determined from public and proprietary databases. The formulation is biased with a focus on appropriate reagent concentrations as well as pH and polymer utilization, but is also balanced to ensure a fair sampling of novel reagents. PEGRx 1 formulations are unique and do not overlap with formulations found in other Hampton Research screens.

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).

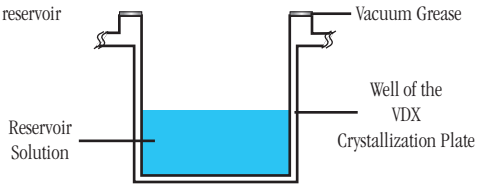
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the PEGRx 1 variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against water although ligands, ions, reducing agents, or other additives should be present as required by the sample for solubility, stability, or activity.

Performing The Screen

The following procedure describes the use of the PEGRx 1 with the Hanging Drop Vapor Diffusion method. The PEGRx 1 is also very compatible with the Sitting Drop, Sandwich Drop, MicroBatch, Microdialysis methods, and Free Interface Diffusion. 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 PEGRx 1. See Figure 1.

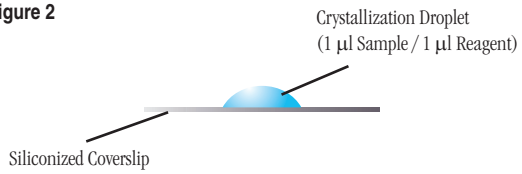
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of PEGRx 1 reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of PEGRx 1 reagent 2 into reservoir A2. Repeat the procedure for the remaining 46 PEGRx 1 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.

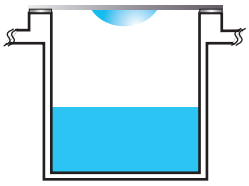
Figure 2



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

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

Figure 3
Inverted siliconized coverslip placed over the reservoir.



6. Repeat operations 3 through 5 for the remaining 47 PEGRx 1 reagents.

7. If the quantity of sample permits, perform the PEGRx 1 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.

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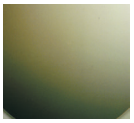
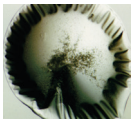
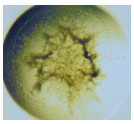
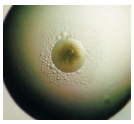
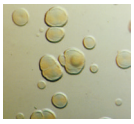
Figure 4
Typical observations in a crystallization experiment

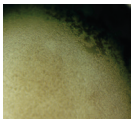

Clear Drop


Skin / Precipitate

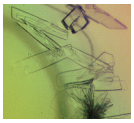

Precipitate


Precipitate / Phase

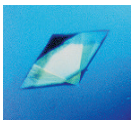

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Interpreting PEGRx 1

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 PEGRx condition and doubling the sample concentration. If more than 33 of the 48 PEGRx 1 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 PEGRx 1 condition. If more than 33 of the 48 PEGRx 1 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, polymer type, polymer concentration, polymer molecular weight, 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.

PEGRx 1 Formulation

PEGRx 1 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).

PEGRx 1 reagents are readily reproduced using Hampton Research Optimize™ stock solutions of polymers and buffers. Optimize stock reagents make reproducing PEGRx 1 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 PEGRx 1. Reagents are combined without further titration.

For further details about formulation, reproducing and optimizing reagents from the PEGRx 1 please refer to PEGRx 1 Fundamentals.

PEGRx 1 reagents are stable at room temperature and are best if used within 12 months of receipt. To enhance reagent stability it is recommended that PEGRx 1 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.

Technical Support

Inquiries regarding PEGRx 1 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 PEGRx 1 Reagents

PEGRx™ 1 reagents and optimization conditions based on PEGRx 1 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 PEGRx 1 reagent 1 in a crystallization plate.
Solution Composition: 0.1 M Citric acid pH 3.5
34% v/v Polyethylene glycol 200

- 560 µl water[3]
- 100 µl 1.0 M Citric acid pH 3.5
(CAS # 77-92-9, Catalog # HR2-757)
- 349 µl 100% Polyethylene glycol 200
(CAS # 25322-68-3, Catalog # HR2-601)

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

Example 2. To prepare 1.0 milliliter of PEGRx 1 reagent 14.
Solution Composition: 0.1 M MES monohydrate pH 6.0
20% v/v Jeffamine® M-600® pH 7.0

- 500 µl water[3]
- 100 µl 1.0 M MES monohydrate pH 6.0
(CAS # 145224-94-8, Catalog # HR2-943-09)
- 400 µl 50% v/v Jeffamine® M-600® pH 7.0
(CAS # 77110-54-4, Catalog # HR2-501)

Make no pH adjustments. Mix well.

Example 3. To prepare 10 milliliters of PEGRx 1 reagent 48.
Solution Composition: 0.1 M BIS-TRIS propane pH 9.0
8% w/v Polyethylene glycol 20,000

- 6.3 ml water[3]
- 1.0 ml 1.0 M BIS-TRIS propane pH 9.0
(CAS # 64431-96-5, Catalog # HR2-993-28)
- 2.7 ml 30% w/v Polyethylene glycol 20,000
(CAS # 25322-68-3, Catalog # HR2-609)

Make no pH adjustments. Mix well.

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

Formulation Notes for PEGRx 1 Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the salts and buffers in Table 1 to reproduce a PEGRx 1 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 Optimize™, Custom Shop™, StockOptions™ pH, and StockOptions™ pH buffer kits from Hampton Research to systematically vary the pH as a crystallization variable.

7. The measured final pH of all PEGRx 1 reagents is available at www.hamptonresearch.com. Search using catalog number HR2-082 and follow the link to the 'PEGRx 1 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 PEGRx 1 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.

Custom Shop™ ready to pipet buffers are made to order, pH titrated buffer stocks from Hampton Research.

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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 PEGRx 1 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).

Polymers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Jeffamine® ED-2001 pH 7.0	HR2-597	50% w/v	200 ml	65605-36-9
Jeffamine® M-600® pH 7.0	HR2-501	50% v/v	200 ml	77110-54-4
Polyethylene glycol 200	HR2-601	100%	200 ml	25322-68-3
Polyethylene glycol 300	HR2-517	100%	200 ml	25322-68-3
Polyethylene glycol 400	HR2-603	100%	200 ml	25322-68-3
Polyethylene glycol 1,000	HR2-523	50% w/v	200 ml	25322-68-3
Polyethylene glycol 1,500	HR2-525	50% w/v	200 ml	25322-68-3
Polyethylene glycol 3,350	HR2-527	50% w/v	200 ml	25322-68-3
Polyethylene glycol 4,000	HR2-529	50% w/v	200 ml	25322-68-3
Polyethylene glycol 6,000	HR2-533	50% w/v	200 ml	25322-68-3
Polyethylene glycol 8,000	HR2-535	50% w/v	200 ml	25322-68-3
Polyethylene glycol 10,000	HR2-607	50% w/v	200 ml	25322-68-3
Polyethylene glycol 20,000	HR2-609	30% w/v	200 ml	25322-68-3
Polyethylene glycol monomethyl ether 550	HR2-611	100%	200 ml	9004-74-4
Polyethylene glycol monomethyl ether 2,000	HR2-613	50% w/v	200 ml	9004-74-4
Polyethylene glycol monomethyl ether 5,000	HR2-615	50% w/v	200 ml	9004-74-4

(Continued on page 3)

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Table 1 (Continued). **Recommended reagents for the formulation of PEGRx 1 and optimization reagents.**

Buffers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
BICINE pH 8.5 [2]	HR2-999-10	1.0 M	185 ml	150-25-4
BIS-TRIS pH 6.5 [1]	HR2-783	1.0 M	100 ml	6976-37-0
BIS-TRIS propane pH 9.0 [1]	HR2-993-28	1.0 M	185 ml	64431-96-5
Citric acid pH 3.5 [2]	HR2-757	1.0 M	100 ml	77-92-9
HEPES pH 7.5 [2]	HR2-729	1.0 M	100 ml	7365-45-9
Imidazole pH 7.0 [1]	HR2-819	1.0 M	100 ml	288-32-4
MES monohydrate pH 6.0 [2]	HR2-943-09	1.0 M	185 ml	145224-94-8
Sodium acetate trihydrate pH 4.0 [1]	HR2-933-05	1.0 M	185 ml	6131-90-4
Sodium acetate trihydrate pH 4.5 [1]	HR2-789	1.0 M	100 ml	6131-90-4
Sodium citrate tribasic dihydrate pH 5.0 [1]	HR2-935-09	1.0 M	185 ml	6132-04-3
Sodium citrate tribasic dihydrate pH 5.5 [1]	HR2-935-14	1.0 M	185 ml	6132-04-3
Tris pH 8.0 [1]	HR2-900-11	1.0 M	185 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 PEGRx 1 and optimization reagents.**

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
StockOptions™ BICINE	HR2-999-**	1.0 M	185 ml	150-25-4	7.6 - 9.0
StockOptions™ Bis-Tris kit [4]	HR2-106	1.0 M	10 ml each	6976-37-0	5.5 - 7.5
StockOptions™ Bis-Tris propane	HR2-993-**	1.0 M	185 ml	64431-96-5	6.3 - 9.5
StockOptions™ Citric acid [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™ Imidazole	HR2-995-**	1.0 M	185 ml	288-32-4	6.2 - 7.8
StockOptions™ MES kit [4]	HR2-243	1.0 M	10 ml each	145224-94-8	5.2 - 7.1
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 [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™					
** Refers to the reagent number in the kit. For example, reagent number 1 = HR2-993-01 (pH 6.3)					

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Tube #	Buffer ◊	Polymer
1.	0.1 M Citric acid pH 3.5	34% v/v Polyethylene glycol 200
2.	0.1 M Sodium citrate tribasic dihydrate pH 5.5	38% v/v Polyethylene glycol 200
3.	0.1 M HEPES pH 7.5	42% v/v Polyethylene glycol 200
4.	0.1 M Sodium acetate trihydrate pH 4.5	30% v/v Polyethylene glycol 300
5.	0.1 M BIS-TRIS pH 6.5	25% v/v Polyethylene glycol 300
6.	0.1 M BICINE pH 8.5	20% v/v Polyethylene glycol 300
7.	0.1 M Sodium acetate trihydrate pH 4.0	15% v/v Polyethylene glycol 400
8.	0.1 M MES monohydrate pH 6.0	22% v/v Polyethylene glycol 400
9.	0.1 M Tris pH 8.0	30% v/v Polyethylene glycol 400
10.	0.1 M Sodium citrate tribasic dihydrate pH 5.0	30% v/v Polyethylene glycol monomethyl ether 550
11.	0.1 M Imidazole pH 7.0	25% v/v Polyethylene glycol monomethyl ether 550
12.	0.1 M BIS-TRIS propane pH 9.0	20% v/v Polyethylene glycol monomethyl ether 550
13.	0.1 M Sodium acetate trihydrate pH 4.0	10% v/v Jeffamine® M-600® pH 7.0
14.	0.1 M MES monohydrate pH 6.0	20% v/v Jeffamine® M-600® pH 7.0
15.	0.1 M Tris pH 8.0	30% v/v Jeffamine® M-600® pH 7.0
16.	0.1 M Citric acid pH 3.5	14% w/v Polyethylene glycol 1,000
17.	0.1 M Sodium citrate tribasic dihydrate pH 5.5	22% w/v Polyethylene glycol 1,000
18.	0.1 M HEPES pH 7.5	30% w/v Polyethylene glycol 1,000
19.	0.1 M Sodium acetate trihydrate pH 4.5	30% w/v Polyethylene glycol 1,500
20.	0.1 M BIS-TRIS pH 6.5	20% w/v Polyethylene glycol 1,500
21.	0.1 M BICINE pH 8.5	15% w/v Polyethylene glycol 1,500
22.	0.1 M Sodium acetate trihydrate pH 4.0	10% w/v Polyethylene glycol monomethyl ether 2,000
23.	0.1 M MES monohydrate pH 6.0	20% w/v Polyethylene glycol monomethyl ether 2,000
24.	0.1 M Tris pH 8.0	30% w/v Polyethylene glycol monomethyl ether 2,000
25.	0.1 M Sodium citrate tribasic dihydrate pH 5.0	30% v/v Jeffamine® ED-2001 pH 7.0
26.	0.1 M Imidazole pH 7.0	20% v/v Jeffamine® ED-2001 pH 7.0
27.	0.1 M BIS-TRIS propane pH 9.0	10% v/v Jeffamine® ED-2001 pH 7.0
28.	0.1 M Citric acid pH 3.5	25% w/v Polyethylene glycol 3,350
29.	0.1 M Sodium citrate tribasic dihydrate pH 5.5	18% w/v Polyethylene glycol 3,350
30.	0.1 M HEPES pH 7.5	12% w/v Polyethylene glycol 3,350
31.	0.1 M Sodium acetate trihydrate pH 4.0	10% w/v Polyethylene glycol 4,000
32.	0.1 M MES monohydrate pH 6.0	14% w/v Polyethylene glycol 4,000
33.	0.1 M Tris pH 8.0	28% w/v Polyethylene glycol 4,000
34.	0.1 M Sodium acetate trihydrate pH 4.5	30% w/v Polyethylene glycol monomethyl ether 5,000
35.	0.1 M BIS-TRIS pH 6.5	20% w/v Polyethylene glycol monomethyl ether 5,000
36.	0.1 M BICINE pH 8.5	8% w/v Polyethylene glycol monomethyl ether 5,000
37.	0.1 M Sodium citrate tribasic dihydrate pH 5.0	10% w/v Polyethylene glycol 6,000
38.	0.1 M Imidazole pH 7.0	20% w/v Polyethylene glycol 6,000
39.	0.1 M BIS-TRIS propane pH 9.0	30% w/v Polyethylene glycol 6,000
40.	0.1 M Citric acid pH 3.5	28% w/v Polyethylene glycol 8,000
41.	0.1 M Sodium citrate tribasic dihydrate pH 5.5	16% w/v Polyethylene glycol 8,000
42.	0.1 M HEPES pH 7.5	4% w/v Polyethylene glycol 8,000
43.	0.1 M Sodium acetate trihydrate pH 4.5	10% w/v Polyethylene glycol 10,000
44.	0.1 M BIS-TRIS pH 6.5	16% w/v Polyethylene glycol 10,000
45.	0.1 M BICINE pH 8.5	20% w/v Polyethylene glycol 10,000
46.	0.1 M Sodium citrate tribasic dihydrate pH 5.0	18% w/v Polyethylene glycol 20,000
47.	0.1 M Imidazole pH 7.0	12% w/v Polyethylene glycol 20,000
48.	0.1 M BIS-TRIS propane pH 9.0	8% w/v Polyethylene glycol 20,000

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

PEGRx™ 1 contains forty-eight unique reagents. To determine the formulation of each reagent, simply read across the page.

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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)

PEGRx™ 1 - HR2-082 Scoring Sheet	Date:	Date:	Date:	Date:
1. 0.1 M Citric acid pH 3.5, 34% v/v Polyethylene glycol 200				
2. 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 38% v/v Polyethylene glycol 200				
3. 0.1 M HEPES pH 7.5, 42% v/v Polyethylene glycol 200				
4. 0.1 M Sodium acetate trihydrate pH 4.5, 30% v/v Polyethylene glycol 300				
5. 0.1 M BIS-TRIS pH 6.5, 25% v/v Polyethylene glycol 300				
6. 0.1 M BICINE pH 8.5, 20% v/v Polyethylene glycol 300				
7. 0.1 M Sodium acetate trihydrate pH 4.0, 15% v/v Polyethylene glycol 400				
8. 0.1 M MES monohydrate pH 6.0, 22% v/v Polyethylene glycol 400				
9. 0.1 M Tris pH 8.0, 30% v/v Polyethylene glycol 400				
10. 0.1 M Sodium citrate tribasic dihydrate pH 5.0, 30% v/v Polyethylene glycol monomethyl ether 550				
11. 0.1 M Imidazole pH 7.0, 25% v/v Polyethylene glycol monomethyl ether 550				
12. 0.1 M BIS-TRIS propane pH 9.0, 20% v/v Polyethylene glycol monomethyl ether 550				
13. 0.1 M Sodium acetate trihydrate pH 4.0, 10% v/v Jeffamine® M-600® pH 7.0				
14. 0.1 M MES monohydrate pH 6.0, 20% v/v Jeffamine® M-600® pH 7.0				
15. 0.1 M Tris pH 8.0, 30% v/v Jeffamine® M-600® pH 7.0				
16. 0.1 M Citric acid pH 3.5, 14% w/v Polyethylene glycol 1,000				
17. 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 22% w/v Polyethylene glycol 1,000				
18. 0.1 M HEPES pH 7.5, 30% w/v Polyethylene glycol 1,000				
19. 0.1 M Sodium acetate trihydrate pH 4.5, 30% w/v Polyethylene glycol 1,500				
20. 0.1 M BIS-TRIS pH 6.5, 20% w/v Polyethylene glycol 1,500				
21. 0.1 M BICINE pH 8.5, 15% w/v Polyethylene glycol 1,500				
22. 0.1 M Sodium acetate trihydrate pH 4.0, 10% w/v Polyethylene glycol monomethyl ether 2,000				
23. 0.1 M MES monohydrate pH 6.0, 20% w/v Polyethylene glycol monomethyl ether 2,000				
24. 0.1 M Tris pH 8.0, 30% w/v Polyethylene glycol monomethyl ether 2,000				
25. 0.1 M Sodium citrate tribasic dihydrate pH 5.0, 30% v/v Jeffamine® ED-2001 pH 7.0				
26. 0.1 M Imidazole pH 7.0, 20% v/v Jeffamine® ED-2001 pH 7.0				
27. 0.1 M BIS-TRIS propane pH 9.0, 10% v/v Jeffamine® ED-2001 pH 7.0				
28. 0.1 M Citric acid pH 3.5, 25% w/v Polyethylene glycol 3,350				
29. 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 18% w/v Polyethylene glycol 3,350				
30. 0.1 M HEPES pH 7.5, 12% w/v Polyethylene glycol 3,350				
31. 0.1 M Sodium acetate trihydrate pH 4.0, 10% w/v Polyethylene glycol 4,000				
32. 0.1 M MES monohydrate pH 6.0, 14% w/v Polyethylene glycol 4,000				
33. 0.1 M Tris pH 8.0, 28% w/v Polyethylene glycol 4,000				
34. 0.1 M Sodium acetate trihydrate pH 4.5, 30% w/v Polyethylene glycol monomethyl ether 5,000				
35. 0.1 M BIS-TRIS pH 6.5, 20% w/v Polyethylene glycol monomethyl ether 5,000				
36. 0.1 M BICINE pH 8.5, 8% w/v Polyethylene glycol monomethyl ether 5,000				
37. 0.1 M Sodium citrate tribasic dihydrate pH 5.0, 10% w/v Polyethylene glycol 6,000				
38. 0.1 M Imidazole pH 7.0, 20% w/v Polyethylene glycol 6,000				
39. 0.1 M BIS-TRIS propane pH 9.0, 30% w/v Polyethylene glycol 6,000				
40. 0.1 M Citric acid pH 3.5, 28% w/v Polyethylene glycol 8,000				
41. 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 16% w/v Polyethylene glycol 8,000				
42. 0.1 M HEPES pH 7.5, 4% w/v Polyethylene glycol 8,000				
43. 0.1 M Sodium acetate trihydrate pH 4.5, 10% w/v Polyethylene glycol 10,000				
44. 0.1 M BIS-TRIS pH 6.5, 16% w/v Polyethylene glycol 10,000				
45. 0.1 M BICINE pH 8.5, 20% w/v Polyethylene glycol 10,000				
46. 0.1 M Sodium citrate tribasic dihydrate pH 5.0, 18% w/v Polyethylene glycol 20,000				
47. 0.1 M Imidazole pH 7.0, 12% w/v Polyethylene glycol 20,000				
48. 0.1 M BIS-TRIS propane pH 9.0, 8% w/v Polyethylene glycol 20,000				

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