PEGRx™ 2

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-084 (pg 1)**

Description

PEGRx™ 2 is a crystallization reagent kit designed to evaluate an array of polymers of varying molecular weight in a medium ionic strength environment in the presence of additives, salts, volatile organics, and polyols versus a wide range of pH. Polymer reagents include Polyethylene glycols and Polyethylene glycol monomethyl ethers. The polymer molecular weight range between 200 and 20,000 is evaluated in a medium 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 secondary reagents which include additives, salts, volatile organics, and polyols. PEGRx 2 is a straightforward, effective, and efficient screen for determining preliminary crystallization conditions in a medium ionic strength, polymeric reagent formulation in the pH range 3.5 to 9. The choice of reagents, reagent 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 2 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).

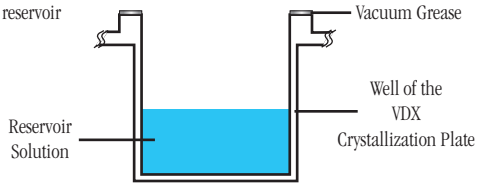
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the PEGRx 2 variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against water although ligands, 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 2 with the Hanging Drop Vapor Diffusion method. The PEGRx 2 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 2. See Figure 1.

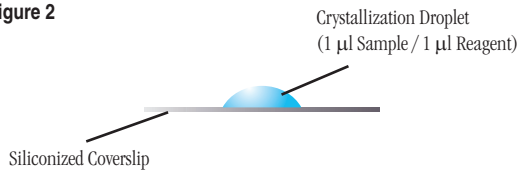
Figure 1
Cross section of a reservoir in the VDX plate.



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

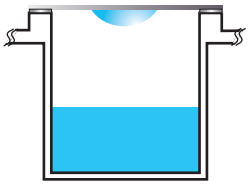
Figure 2



4. Pipet 1 µl of PEGRx 2 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 2 reagents.

7. If the quantity of sample permits, perform the PEGRx 2 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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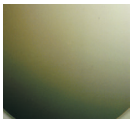
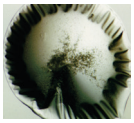
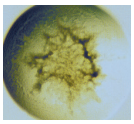
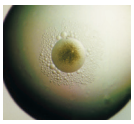
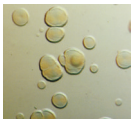
Figure 4
Typical observations in a crystallization experiment

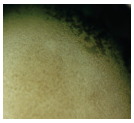

Clear Drop


Skin / Precipitate

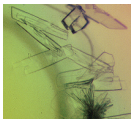

Precipitate


Precipitate / Phase

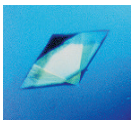

Quasi Crystals


Microcrystals

Needle Cluster


Plates

Rod Cluster


Single Crystal

Interpreting PEGRx 2

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 2 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 2 condition. If more than 33 of the 48 PEGRx 2 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 2 Formulation

PEGRx 2 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 2 reagents are readily reproduced using Hampton Research Optimize™ stock solutions of polymers and buffers. Optimize stock reagents make reproducing PEGRx 2 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 2. Reagents are combined without further titration.

For further details about formulation, reproducing and optimizing reagents from the PEGRx 2 please refer to PEGRx 2 Fundamentals.

PEGRx 2 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 recommended that PEGRx 2 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 2 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 2 Reagents

PEGRx™ 2 reagents and optimization conditions based on PEGRx 2 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 2 reagent 1 in a crystallization plate.
Solution Composition: 0.8 M Lithium sulfate monohydrate
0.1 M Sodium acetate trihydrate pH 4.0
4% v/v Polyethylene glycol 200

- 460 µl water[3]
- 400 µl 2.0 M Lithium sulfate monohydrate (CAS # 10102-25-7, Catalog # HR2-545)
- 100 µl 1.0 M Sodium acetate trihydrate pH 4.0 (CAS # 6131-90-4, Catalog # HR2-933-05)
- 40 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 2 reagent 15.
Solution Composition: 0.1 M Sodium malonate pH 8.0
0.1 M Tris pH 8.0
30% w/v Polyethylene glycol 1,000

- 570.6 µl water[3]
- 29.4 µl 3.4 M Sodium malonate pH 8.0 (CAS # 141-82-2, Catalog # HR2-807)
- 100 µl 1.0 M Tris pH 8.0 (CAS # 77-86-1, Catalog # HR2-900-11)
- 300 µl 100% Polyethylene glycol 1,000 (CAS # 25322-68-3, Catalog # HR2-523)

Make no pH adjustments. Mix well.

Example 3. To prepare 1 milliliter of PEGRx 2 reagent 48.
Solution Composition: 3% w/v Dextran sulfate sodium salt
0.1 M BICINE pH 8.5
15% w/v Polyethylene glycol 20,000

- 300 µl water[3]
- 100 µl 1.0 M BICINE pH 8.5 (CAS # 150-25-4, Catalog # HR2-999-10)
- 100 µl 30% w/v Dextran sulfate sodium salt (CAS # 9011-18-1, Catalog # HR2-428-51)
- 500 µl 30% w/v Polyethylene glycol 20,000 (CAS # 25322-68-3, Catalog # HR2-609)

Make no pH adjustments. Mix well.

[3] ASTM Type I water.

Formulation Notes for PEGRx 2 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 2 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 2 reagents is available at www.hamptonresearch.com. Search using catalog number HR2-084 and follow the link to the 'PEGRx 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 PEGRx 2 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 2 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).

Additive	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Dextran sulfate sodium salt	HR2-428-51	30% w/v	1.0 ml	9011-18-1
n-Octyl-β-D-glucoside	HR2-428-71	5% w/v	1.0 ml	29836-26-8
Organics (Volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
1,4-Dioxane	HR2-617	100%	200 ml	123-91-1
2-Propanol	HR2-619	100%	200 ml	67-63-0
Polyol	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100%	200 ml	107-41-5
Ethylene glycol	HR2-621	100%	100 ml	107-21-1
Polyethylene glycol 200	HR2-601	100%	200 ml	25322-68-3
Polyethylene glycol 400	HR2-603	100%	200 ml	25322-68-3
Salt	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
Ammonium acetate	HR2-565	1.0 M	100 ml	631-61-8
	HR2-799	8.0 M	200 ml	631-61-8

(Salt Continued on page 3)

Table 1 (Continued). **Recommended reagents for the formulation of PEGRx 2 and optimization reagents.**

Salt	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 sulfate	HR2-541	3.5 M	200 ml	7783-20-2
Cadmium chloride hydrate	HR2-715	1.0 M	100 ml	654054-66-7
Calcium chloride dihydrate	HR2-557	2.0 M	100 ml	10035-04-8
L-Proline	HR2-775	1.0 M	100 ml	147-85-3
Lithium sulfate monohydrate	HR2-545	2.0 M	200 ml	10377-48-7
Magnesium chloride hexahydrate	HR2-559	2.0 M	100 ml	7791-18-6
	HR2-803	5.0 M	200 ml	7791-18-6
Magnesium formate dihydrate	HR2-537	1.0 M	200 ml	557-39-1
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	200 ml	7791-20-0
Potassium formate	HR2-667	14.0 M	200 ml	590-29-4
Potassium sodium tartrate tetrahydrate	HR2-539	1.5 M	200 ml	6381-59-5
Succinic acid pH 7.0	HR2-709	1.2 M	200 ml	110-15-6
Sodium chloride	HR2-637	5.0 M	200 ml	7647-14-5
Sodium formate	HR2-547	7.0 M	200 ml	141-53-7
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 8.0	HR2-807	3.4 M	200 ml	141-82-2
Tacsimate pH 4.0	HR2-823	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
Polymer	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
Polyethylene glycol 200	HR2-601	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

(Polymer Continued on page 4)

Table 1 (Continued). Recommended reagents for the formulation of PEGRx 2 and optimization reagents.

Polymer	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
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
Buffer	**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 2 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

(Buffer Solution or Kit Continued on page 5)

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Table 2. Recommended buffers for screening the pH of PEGRx 2 and optimization reagents.

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
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)

Technical Support

Inquiries regarding PEGRx 2 Fundamentals, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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Tube #	Additive / Salt / Volatile Organic / Polyol	Tube #	Buffer ◊	Tube #	Polymer
1.	0.8 M Lithium sulfate monohydrate	1.	0.1 M Sodium acetate trihydrate pH 4.0	1.	4% v/v Polyethylene glycol 200
2.	0.2 M Lithium sulfate monohydrate	2.	0.1 M Sodium citrate tribasic dihydrate pH 5.0	2.	26% v/v Polyethylene glycol 200
3.	0.05 M Calcium chloride dihydrate	3.	0.1 M MES monohydrate pH 6.0	3.	45% v/v Polyethylene glycol 200
4.	28% v/v 2-Propanol	4.	0.1 M BIS-TRIS pH 6.5	4.	3% v/v Polyethylene glycol 200
5.	20% v/v Tacsimate pH 7.0	5.	0.1 M HEPES pH 7.5	5.	2% v/v Polyethylene glycol 200
6.	10% v/v 2-Propanol	6.	0.1 M Sodium citrate tribasic dihydrate pH 5.0	6.	26% v/v Polyethylene glycol 400
7.	0.2 M Ammonium acetate	7.	0.1 M Sodium citrate tribasic dihydrate pH 5.5	7.	24% v/v Polyethylene glycol 400
8.	0.2 M Ammonium sulfate	8.	0.1 M BIS-TRIS pH 6.5	8.	18% v/v Polyethylene glycol 400
9.		9.	0.1 M HEPES pH 7.5	9.	40% v/v Polyethylene glycol 400
10.	6% v/v 2-Propanol	10.	0.1 M Sodium acetate trihydrate pH 4.5	10.	26% v/v Polyethylene glycol monomethyl ether 550
11.	1.8 M Ammonium sulfate	11.	0.1 M BIS-TRIS pH 6.5	11.	2% v/v Polyethylene glycol monomethyl ether 550
12.	0.15 M DL-Malic acid pH 7.0	12.	0.1 M Imidazole pH 7.0	12.	22% v/v Polyethylene glycol monomethyl ether 550
13.	0.1 M Succinic acid pH 7.0	13.	0.1 M BICINE pH 8.5	13.	30% v/v Polyethylene glycol monomethyl ether 550
14.	0.1 M Lithium sulfate monohydrate	14.	0.1 M Sodium citrate tribasic dihydrate pH 5.5	14.	20% w/v Polyethylene glycol 1,000
15.	0.1 M Sodium malonate pH 8.0	15.	0.1 M Tris pH 8.0	15.	30% w/v Polyethylene glycol 1,000
16.	4% v/v (+/-)-2-Methyl-2,4-pentanediol	16.	0.1 M Citric acid pH 3.5	16.	20% w/v Polyethylene glycol 1,500
17.	0.2 M L-Proline	17.	0.1 M HEPES pH 7.5	17.	24% w/v Polyethylene glycol 1,500
18.	10% v/v 2-Propanol	18.	0.1 M BICINE pH 8.5	18.	30% w/v Polyethylene glycol 1,500
19.	0.1 M Sodium chloride	19.	0.1 M BIS-TRIS propane pH 9.0	19.	25% w/v Polyethylene glycol 1,500
20.	0.02 M Nickel(II) chloride hexahydrate, 0.02 M Magnesium chloride hexahydrate, 0.02 M Cadmium chloride hydrate	20.	0.1 M Sodium acetate trihydrate pH 4.5	20.	24% w/v Polyethylene glycol monomethyl ether 2,000
21.	20% v/v 2-Propanol	21.	0.1 M MES monohydrate pH 6.0	21.	20% w/v Polyethylene glycol monomethyl ether 2,000
22.	0.2 M Ammonium citrate tribasic pH 7.0	22.	0.1 M Imidazole pH 7.0	22.	20% w/v Polyethylene glycol monomethyl ether 2,000
23.	4.0 M Potassium formate	23.	0.1 M BIS-TRIS propane pH 9.0	23.	2% w/v Polyethylene glycol monomethyl ether 2,000
24.	50% v/v Tacsimate pH 4.0	24.	0.1 M Sodium acetate trihydrate pH 4.5	24.	1% w/v Polyethylene glycol 3,350
25.	0.10% w/v n-Octyl-β-D-glucoside	25.	0.1 M Sodium citrate tribasic dihydrate pH 5.5	25.	22% w/v Polyethylene glycol 3,350
26.	2% v/v Tacsimate pH 7.0, 5% v/v 2-Propanol	26.	0.1 M Imidazole pH 7.0	26.	8% w/v Polyethylene glycol 3,350
27.	2% v/v 1,4-Dioxane	27.	0.1 M Tris pH 8.0	27.	15% w/v Polyethylene glycol 3,350
28.	18% v/v 2-Propanol	28.	0.1 M Sodium citrate tribasic dihydrate pH 5.5	28.	20% w/v Polyethylene glycol 4,000
29.	6% v/v Tacsimate pH 6.0	29.	0.1 M MES monohydrate pH 6.0	29.	25% w/v Polyethylene glycol 4,000
30.	0.2 M Magnesium formate dihydrate	30.	0.1 M Sodium acetate trihydrate pH 4.0	30.	18% w/v Polyethylene glycol monomethyl ether 5,000
31.	2% v/v Polyethylene glycol 400	31.	0.1 M Imidazole pH 7.0	31.	24% w/v Polyethylene glycol monomethyl ether 5,000
32.	0.2 M Sodium formate	32.	0.1 M BICINE pH 8.5	32.	20% w/v Polyethylene glycol monomethyl ether 5,000
33.	4% v/v 2-Propanol	33.	0.1 M BIS-TRIS propane pH 9.0	33.	20% w/v Polyethylene glycol monomethyl ether 5,000
34.	6% v/v Ethylene glycol	34.	0.1 M Citric acid pH 3.5	34.	10% w/v Polyethylene glycol 6,000
35.	0.15 M Lithium sulfate monohydrate	35.	0.1 M Citric acid pH 3.5	35.	18% w/v Polyethylene glycol 6,000
36.	10% v/v 2-Propanol	36.	0.1 M Sodium acetate trihydrate pH 4.0	36.	22% w/v Polyethylene glycol 6,000
37.	0.2 M Sodium chloride	37.	0.1 M Sodium acetate trihydrate pH 4.0	37.	22% w/v Polyethylene glycol 8,000
38.	20% v/v 2-Propanol	38.	0.1 M Tris pH 8.0	38.	5% w/v Polyethylene glycol 8,000
39.	10% v/v Polyethylene glycol 200	39.	0.1 M BIS-TRIS propane pH 9.0	39.	18% w/v Polyethylene glycol 8,000
40.	15% v/v 2-Propanol	40.	0.1 M Sodium citrate tribasic dihydrate pH 5.0	40.	10% w/v Polyethylene glycol 10,000
41.	0.4 M Sodium malonate pH 6.0	41.	0.1 M MES monohydrate pH 6.0	41.	0.5% w/v Polyethylene glycol 10,000
42.	0.2 M Potassium sodium tartrate tetrahydrate	42.	0.1 M BIS-TRIS pH 6.5	42.	10% w/v Polyethylene glycol 10,000
43.	5% v/v (+/-)-2-Methyl-2,4-pentanediol	43.	0.1 M HEPES pH 7.5	43.	10% w/v Polyethylene glycol 10,000
44.	0.2 M Ammonium acetate	44.	0.1 M Tris pH 8.0	44.	16% w/v Polyethylene glycol 10,000
45.	5% v/v 2-Propanol	45.	0.1 M Citric acid pH 3.5	45.	6% w/v Polyethylene glycol 20,000
46.	1.0 M Sodium malonate pH 5.0	46.	0.1 M Sodium acetate trihydrate pH 4.5	46.	2% w/v Polyethylene glycol 20,000
47.	0.2 M Magnesium chloride hexahydrate	47.	0.1 M Sodium citrate tribasic dihydrate pH 5.0	47.	10% w/v Polyethylene glycol 20,000
48.	3% w/v Dextran sulfate sodium salt	48.	0.1 M BICINE pH 8.5	48.	15% 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™ 2 contains forty-eight unique reagents. To determine the formulation of each reagent, simply read across the page.

34 Journey
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HAMPTON RESEARCH
Solutions for Crystal Growth

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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)

PEGRx™ 2 - HR2-084 Scoring Sheet

	Date:	Date:	Date:
1. 0.8 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.0, 4% v/v Polyethylene glycol 200			
2. 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.0, 26% v/v Polyethylene glycol 200			
3. 0.05 M Calcium chloride dihydrate, 0.1 M MES monohydrate pH 6.0, 45% v/v Polyethylene glycol 200			
4. 28% v/v 2-Propanol, 0.1 M BIS-TRIS pH 6.5, 3% v/v Polyethylene glycol 200			
5. 20% v/v Tacsimate pH 7.0, 0.1 M HEPES pH 7.5, 2% v/v Polyethylene glycol 200			
6. 10% v/v 2-Propanol, 0.1 M Sodium citrate tribasic dihydrate pH 5.0, 26% v/v Polyethylene glycol 400			
7. 0.2 M Ammonium acetate, 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 24% v/v Polyethylene glycol 400			
8. 0.2 M Ammonium sulfate, 0.1 M BIS-TRIS pH 6.5, 18% v/v Polyethylene glycol 400			
9. 0.1 M HEPES pH 7.5, 40% v/v Polyethylene glycol 400			
10. 6% v/v 2-Propanol, 0.1 M Sodium acetate trihydrate pH 4.5, 26% v/v Polyethylene glycol monomethyl ether 550			
11. 1.8 M Ammonium sulfate, 0.1 M BIS-TRIS pH 6.5, 2% v/v Polyethylene glycol monomethyl ether 550			
12. 0.15 M DL-Malic acid pH 7.0, 0.1 M Imidazole pH 7.0, 22% v/v Polyethylene glycol monomethyl ether 550			
13. 0.1 M Succinic acid pH 7.0, 0.1 M BICINE pH 8.5, 30% v/v Polyethylene glycol monomethyl ether 550			
14. 0.1 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 20% w/v Polyethylene glycol 1,000			
15. 0.1 M Sodium malonate pH 8.0, 0.1 M Tris pH 8.0, 30% w/v Polyethylene glycol 1,000			
16. 4% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.1 M Citric acid pH 3.5, 20% w/v Polyethylene glycol 1,500			
17. 0.2 M L-Proline, 0.1 M HEPES pH 7.5, 24% w/v Polyethylene glycol 1,500			
18. 10% v/v 2-Propanol, 0.1 M BICINE pH 8.5, 30% w/v Polyethylene glycol 1,500			
19. 0.1 M Sodium chloride, 0.1 M BIS-TRIS propane pH 9.0, 25% w/v Polyethylene glycol 1,500			
20. 0.02 M Nickel(II) chloride hexahydrate, 0.02 M Magnesium chloride hexahydrate, 0.02 M Cadmium chloride hydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 24% w/v Polyethylene glycol monomethyl ether 2,000			
21. 20% v/v 2-Propanol, 0.1 M MES monohydrate pH 6.0, 20% w/v Polyethylene glycol monomethyl ether 2,000			
22. 0.2 M Ammonium citrate tribasic pH 7.0, 0.1 M Imidazole pH 7.0, 20% w/v Polyethylene glycol monomethyl ether 2,000			
23. 4.0 M Potassium formate, 0.1 M BIS-TRIS propane pH 9.0, 2% w/v Polyethylene glycol monomethyl ether 2,000			
24. 50% v/v Tacsimate pH 4.0, 0.1 M Sodium acetate trihydrate pH 4.5, 1% w/v Polyethylene glycol 3,350			
25. 0.10% w/v n-Octyl-β-D-glucoside, 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 22% w/v Polyethylene glycol 3,350			
26. 2% v/v Tacsimate pH 7.0, 5% v/v 2-Propanol, 0.1 M Imidazole pH 7.0, 8% w/v Polyethylene glycol 3,350			
27. 2% v/v 1,4-Dioxane, 0.1 M Tris pH 8.0, 15% w/v Polyethylene glycol 3,350			
28. 18% v/v 2-Propanol, 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 20% w/v Polyethylene glycol 4,000			
29. 6% v/v Tacsimate pH 6.0, 0.1 M MES monohydrate pH 6.0, 25% w/v Polyethylene glycol 4,000			
30. 0.2 M Magnesium formate dihydrate, 0.1 M Sodium acetate trihydrate pH 4.0, 18% w/v Polyethylene glycol monomethyl ether 5,000			
31. 2% v/v Polyethylene glycol 400, 0.1 M Imidazole pH 7.0, 24% w/v Polyethylene glycol monomethyl ether 5,000			
32. 0.2 M Sodium formate, 0.1 M BICINE pH 8.5, 20% w/v Polyethylene glycol monomethyl ether 5,000			
33. 4% v/v 2-Propanol, 0.1 M BIS-TRIS propane pH 9.0, 20% w/v Polyethylene glycol monomethyl ether 5,000			
34. 6% v/v Ethylene glycol, 0.1 M Citric acid pH 3.5, 10% w/v Polyethylene glycol 6,000			
35. 0.15 M Lithium sulfate monohydrate, 0.1 M Citric acid pH 3.5, 18% w/v Polyethylene glycol 6,000			
36. 10% v/v 2-Propanol, 0.1 M Sodium acetate trihydrate pH 4.0, 22% w/v Polyethylene glycol 6,000			
37. 0.2 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.0, 22% w/v Polyethylene glycol 8,000			
38. 20% v/v 2-Propanol, 0.1 M Tris pH 8.0, 5% w/v Polyethylene glycol 8,000			
39. 10% v/v Polyethylene glycol 200, 0.1 M BIS-TRIS propane pH 9.0, 18% w/v Polyethylene glycol 8,000			
40. 15% v/v 2-Propanol, 0.1 M Sodium citrate tribasic dihydrate pH 5.0, 10% w/v Polyethylene glycol 10,000			
41. 0.4 M Sodium malonate pH 6.0, 0.1 M MES monohydrate pH 6.0, 0.5% w/v Polyethylene glycol 10,000			
42. 0.2 M Potassium sodium tartrate tetrahydrate, 0.1 M BIS-TRIS pH 6.5, 10% w/v Polyethylene glycol 10,000			
43. 5% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 10,000			
44. 0.2 M Ammonium acetate, 0.1 M Tris pH 8.0, 16% w/v Polyethylene glycol 10,000			
45. 5% v/v 2-Propanol, 0.1 M Citric acid pH 3.5, 6% w/v Polyethylene glycol 20,000			
46. 1.0 M Sodium malonate pH 5.0, 0.1 M Sodium acetate trihydrate pH 4.5, 2% w/v Polyethylene glycol 20,000			
47. 0.2 M Magnesium chloride hexahydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.0, 10% w/v Polyethylene glycol 20,000			
48. 3% w/v Dextran sulfate sodium salt, 0.1 M BICINE pH 8.5, 15% w/v Polyethylene glycol 20,000			

Solutions for Crystal Growth

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