MembFac™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-114 (pg 1)**

MembFac™ is a complete reagent kit designed to provide a rapid screening method for the crystallization of membrane proteins as well as biological macromolecules. MembFac is a straightforward, effective, and practical kit for determining preliminary crystallization conditions. MembFac is also effective in determining the solubility of a macromolecule in a wide range of precipitants and pH.

The kit is designed to provide a sparse matrix of trial conditions selected from known and published crystallization conditions. The reagent parameter variables are pH, buffer material, salt, and precipitant. Five different pH's: 4.6, 5.6, 6.5, 7.5, and 8.5 are utilized with Sodium acetate, Sodium citrate, ADA, HEPES sodium, and TRIS hydrochloride as the respective buffers. The four categories of precipitating agents utilized are volatile agents, non-volatile agents, salts, and a combination of these three. Refer to the enclosed MembFac reagent formulation for additional information.

Sample Preparation

The membrane protein of interest is isolated in the detergent which gives the highest stability/activity ratio. The final protein concentration should be 10 to 20 mg/ml and the detergent concentration should only be slightly above the CMC.

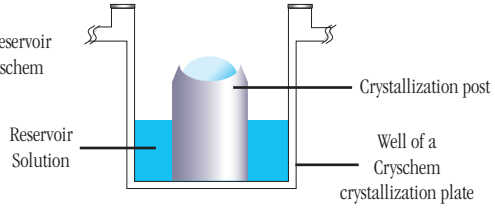
The sample should be as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation prior to use.

Performing The Screen

The following procedure describes the use of MembFac with the Sitting Drop Vapor Diffusion method. MembFac is also very compatible with the Hanging Drop method as well. 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. Using a Cryschem Plate for Sitting Drop Vapor Diffusion (HR3-160) and using a clean pipet tip, pipet 1 ml of MembFac reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of MembFac reagent 2 into reservoir A2. Repeat the procedure for the remaining 46 MembFac reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over. See Figure 1.

Figure 1
Cross section of a reservoir and post in the Cryschem plate.

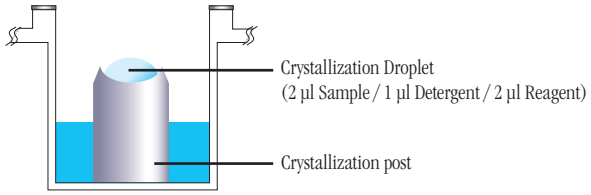


2. Pipet 2 µl of the sample to the center of the crystallization post of the Cryschem plate. See Figure 2.

3. One may choose to pipet the detergent directly into the sample, or dilute the detergent in the reservoir and then pipet the reservoir containing the detergent into the drop. In either situation, the crystallization screening detergent concentration in the drop, prior to equilibration with the reservoir should be 1 to 3 times the CMC.

4. When placing the detergent directly into the drop, pipet 1 µl of the selected crystallization screening detergent (suggested stock detergent concentration equal to ten times the CMC) into the 2 µl sample drop. See Figure 2.

Figure 2



5. Pipet 2 µl of MembFac 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.

6. Repeat operations 4 and 5 for the remaining 47 MembFac reagents.

7. Seal the remaining reagent in the Deep Well block using either sealing tape, film, or cap mat.

8. If the quantity of sample permits, perform MembFac 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.

Detergent Considerations

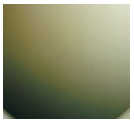
One will need to set one MembFac Screen for each crystallization detergent to be screened. It is recommended that one begin screening crystallization detergents with a larger CMC and work toward detergents with a smaller CMC. For example, one might work in the following series: n-hexyl-β-D-glucoside, Zwittergent® 3-10, n-Octyl-β-D-glucoside, nonyl-β-D-glucoside, LDAO, Cymal®-6, and $C_{12}E_8$.

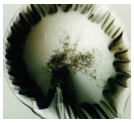
One convenient method for screening crystallization detergents is to utilize the Hampton Research Detergent Screening Kits with 96 crystallization detergents preformulated at 10 times the CMC for each detergent (Catalog Number: HR2-406 & HR2-408).

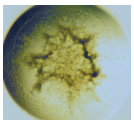
Examine The Drop

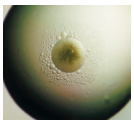
Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after. Records

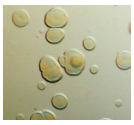
Figure 3
Typical observations in a crystallization experiment

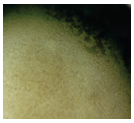

Clear Drop


Skin / Precipitate

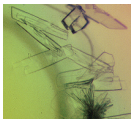

Precipitate


Precipitate / Phase

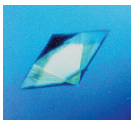

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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 3 (on the left side of page 2) shows typical examples of what one might observe in a crystallization experiment.

Interpreting MembFac

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 MembFac condition and doubling the sample concentration. If more than 33 of the 48 MembFac 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 MembFac condition. If more than 33 of the 48 MembFac 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.

Other considerations include the screening of alternate crystallization detergents with unique CMC's, MW, and head groups, and include small amphiphiles in the drop to manipulate sample-sample, sample-solvent, sample-detergent, and detergent-detergent interactions. Consider 0.5 to 1.0% of 1,2,3-Heptanetriol, MPD, benzamidine HCl, 1,6-Hexanediol, Ethylene glycol, 1,2-Dimethoxyethane, or CTAB as amphiphiles.

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.

MembFac Formulation

MembFac 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).

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

MembFac reagents containing buffers are formulated by creating a 1.0 M stock buffer, titrated to the desired pH using hydrochloric acid or sodium hydroxide. The buffer is then diluted with the other reagent components and water. No further pH adjustment is required.

MembFac 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 MembFac 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 MembFac 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.

MembFac™

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References and Readings

1. Garavito, R.M. et al, J. Crystal Growth, 765, 701-709, 1986.
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3. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology Academic Press. Volume 1, Number 1, August 1990.
4. Crystallization of membrane proteins. Edited by Hartmut Michel, CRC Press, 1991.
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6. Crystallization of nucleic acids and proteins. Edited by A. Ducruix and R. Giege. The Practical Approach Series. Oxford Univ. Press 1992.
7. Cudney, B. et al, Acta Cryst, D50, 414-423, (1994).

Technical Support

Inquiries regarding MembFac 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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MembFac™

HAMPTON RESEARCH
Solutions for Crystal Growth

MembFac Fundamentals HR2-114

How to Reproduce MembFac Reagents

MembFac reagents and optimization conditions based on MembFac 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 MembFac reagent 1 in a crystallization plate.

Solution Composition: 0.1 M Sodium chloride
0.1 M Sodium acetate trihydrate pH 4.6
12% v/v (+/-)-2-Methyl-2,4-pentanediol

- 720 µl water[3]
- 100 µl 1.0 M Sodium acetate trihydrate pH 4.6 (CAS # 6131-90-4, Catalog # HR2-731)
- 20 µl 5.0 M Sodium chloride (CAS # 7647-14-5, Catalog # HR2-637)
- 120 µl 100% (+/-)-2-Methyl-2,4-pentanediol (CAS # 107-41-5, Catalog # HR2-627)

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

Example 2. To prepare 1.0 milliliter of MembFac reagent 34.
Solution Composition: 0.1 M Ammonium sulfate
0.1 M HEPES sodium pH 7.5
10% w/v Polyethylene glycol 4,000

- 671 µl water[3]
- 29 µl 3.5 M Ammonium sulfate (CAS # 7783-20-2, Catalog # HR2-541)
- 100 µl 1.0 M HEPES sodium pH 7.5 (CAS # 75277-39-3, Catalog # HR2-733)
- 200 µl 50% w/v Polyethylene glycol 4,000 (CAS # 25322-68-3, Catalog # HR2-529)

Make no pH adjustments. Mix well.

Example 3. To prepare 10 milliliters of MembFac reagent 14.

Solution Composition: 0.1 M Sodium citrate tribasic dihydrate
0.1 M Sodium citrate tribasic dihydrate pH 5.6
10% v/v 2-Propanol

- 7.4 ml water[3]
- 0.6 ml 1.6 M Sodium citrate tribasic dihydrate (CAS # 6132-04-3, Catalog # HR2-549)
- 1.0 ml 1.0 M Sodium citrate tribasic dihydrate pH 5.6 (CAS # 6132-04-3, Catalog # HR2-735)
- 1.0 ml 100% 2-Propanol (CAS # 67-63-0, Catalog # HR2-619)

Make no pH adjustments. Mix well.

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

Formulation Notes for MembFac Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce an MembFac reagent.
2. All Optimize solutions and screen reagents are sterile filtered using 0.22 µm filters into sterile containers.
3. Add water first as this will help maintain the solubility of subsequently added reagents.
4. When formulating reagents using a pipet, add the largest volume last (except water). Use this larger volume setting to aspirate and dispense the reagent until the solution is mixed.
5. When formulating reagents using a pipet, use a clean, sterile pipet tip for each reagent added to the solution.
6. Use the buffers in Table 2 to systematically vary the pH as a crystallization variable.

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 an MembFac kit.

Optimize™ buffer stocks are supplied as a 100 milliliters sterile filtered solution. Optimize buffers are available as an acid-base pair or titrated to a specific pH.

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.

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 MembFac and Optimization reagents.

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 provides the common chemical name, the Hampton Research catalog number, supplied stock concentration, the supplied volume, and the CAS number for each reagent. For more information on a specific Optimize reagent, go to www.hamptonresearch.com. Using Search, enter either the catalog number, CAS number, or chemical name to obtain additional information for the Optimize reagent, including a Certificate of Analysis and MSDS (where applicable).

Salts	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Ammonium phosphate monobasic	HR2-555	2.5 M	200 ml	7722-76-1
Ammonium phosphate dibasic	HR2-629	3.5 M	200 ml	7783-28-0
Ammonium sulfate	HR2-541	3.5 M	200 ml	7783-20-2
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 sulfate heptahydrate	HR2-821	2.0 M	200 ml	10034-99-8
Magnesium sulfate hydrate	HR2-633	2.5 M	200 ml	22189-08-8
Potassium phosphate dibasic	HR2-635	4.0 M	200 ml	7758-11-4
Potassium sodium tartrate tetrahydrate	HR2-539	1.5 M	200 ml	6381-59-5
Sodium acetate trihydrate	HR2-543	3.0 M	200 ml	6131-90-4
Sodium chloride	HR2-637	5.0 M	200 ml	7647-14-5
Sodium citrate tribasic dihydrate	HR2-549	1.6 M	200 ml	6132-04-3
Sodium phosphate dibasic dihydrate	HR2-639	1.0 M	200 ml	10028-24-7
Zinc acetate dihydrate	HR2-563	1.0 M	100 ml	5970-45-6
Polymers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
Polyethylene glycol 400	HR2-603	100 %	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

(Continued on page 3)

Table 1 (Continued). **Recommended reagents for the formulation of MembFac and Optimization reagents.**

Organics (non-volatile)	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100 %	200 ml	107-41-5
Organics (non-volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
2-Propanol	HR2-619	100%	200 ml	67-63-0
Buffers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
ADA pH 6.5 [2]	HR2-817	1.0 M	100 ml	26239-55-4
HEPES sodium pH 7.5 [1]	HR2-733	1.0 M	100 ml	75277-39-3
Sodium acetate trihydrate pH 4.6 [1]	HR2-731	1.0 M	100 ml	6131-90-4
Sodium citrate tribasic dihydrate pH 5.6 [1]	HR2-735	1.0 M	100 ml	6132-04-3
Tris hydrochloride pH 8.5 [2]	HR2-727	1.0 M	100 ml	1185-53-1
[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 MembFac and Optimization reagents.**

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
ADA untitrated	HR2-507	0.5 M	100 ml	26239-55-4	5.6 - 7.5
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
HEPES sodium untitrated	HR2-577	1.0 M	100 ml	75277-39-3	6.6 - 8.5
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Hepes kit [4]	HR2-231	1.0 M	10 ml each	75277-39-3	6.8 - 8.2
Sodium acetate trihydrate untitrated	HR2-569	1.0 M	100 ml	6131-90-4	3.6 - 5.6
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Acetate kit [4]	HR2-233	1.0 M	10 ml each	6131-90-4	3.6 - 5.6
Sodium citrate tribasic dihydrate untitrated	HR2-571	1.0 M	100 ml	6132-04-3	3.0 - 6.2
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Citrate kit [4]	HR2-235	1.0 M	10 ml each	6132-04-3	4.2 - 6.5

(Continued on page 4)

Table 2. Recommended buffers for screening the pH of MembFac and Optimization reagents.

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
Tris hydrochloride untitrated	HR2-579	1.0 M	100 ml	1185-53-1	7.0 - 9.0
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ Tris Hydrochloride kit [4]	HR2-237	1.0 M	10 ml each	1185-53-1	7.0 - 9.0

[4] Individual StockOptions buffers titrated to any pH within the kit's pH range are available in 185 ml volumes from the Hampton Research Custom Shop

Technical Support

Inquiries regarding MembFac 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 #	Salt	Buffer ◊	Precipitant
1.	0.1 M Sodium chloride	0.1 M Sodium acetate trihydrate pH 4.6	12% v/v (+/-)-2-Methyl-2,4-pentanediol
2.	0.1 M Zinc acetate dihydrate	0.1 M Sodium acetate trihydrate pH 4.6	12% w/v Polyethylene glycol 4,000
3.	0.2 M Ammonium sulfate	0.1 M Sodium acetate trihydrate pH 4.6	10% w/v Polyethylene glycol 4,000
4.	0.1 M Sodium chloride	0.1 M Sodium acetate trihydrate pH 4.6	12% v/v 2-Propanol
5.	None	0.1 M Sodium acetate trihydrate pH 4.6	12% w/v Polyethylene glycol 4,000
6.	None	0.1 M Sodium acetate trihydrate pH 4.6	1.0 M Ammonium sulfate
7.	None	0.1 M Sodium acetate trihydrate pH 4.6	1.0 M Magnesium sulfate heptahydrate
8.	0.1 M Magnesium chloride hexahydrate	0.1 M Sodium acetate trihydrate pH 4.6	18% v/v Polyethylene glycol 400
9.	0.1 M Lithium sulfate monohydrate	0.1 M Sodium acetate trihydrate pH 4.6	1.0 M Ammonium phosphate monobasic
10.	0.1 M Sodium chloride	0.1 M Sodium acetate trihydrate pH 4.6	12% w/v Polyethylene glycol 6,000
11.	0.1 M Magnesium chloride hexahydrate	0.1 M Sodium acetate trihydrate pH 4.6	12% w/v Polyethylene glycol 6,000
12.	0.1 M Sodium chloride	0.1 M Sodium citrate tribasic dihydrate pH 5.6	18% v/v Polyethylene glycol 400
13.	0.1 M Lithium sulfate monohydrate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	12% w/v Polyethylene glycol 4,000
14.	0.1 M Sodium citrate tribasic dihydrate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	10% v/v 2-Propanol
15.	0.1 M Sodium chloride	0.1 M Sodium citrate tribasic dihydrate pH 5.6	12% v/v (+/-)-2-Methyl-2,4-pentanediol
16.	None	0.1 M Sodium citrate tribasic dihydrate pH 5.6	1.0 M Magnesium sulfate heptahydrate
17.	0.1 M Sodium chloride	0.1 M Sodium citrate tribasic dihydrate pH 5.6	12% w/v Polyethylene glycol 4,000
18.	0.1 M Lithium sulfate monohydrate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	12% w/v Polyethylene glycol 6,000
19.	0.1 M Magnesium chloride hexahydrate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	4% v/v (+/-)-2-Methyl-2,4-pentanediol
20.	None	0.1 M Sodium citrate tribasic dihydrate pH 5.6	0.1 M Sodium chloride
21.	0.1 M Lithium sulfate monohydrate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	4% v/v Polyethylene glycol 400
22.	None	0.1 M ADA pH 6.5	1.0 M Ammonium sulfate
23.	0.1 M Lithium sulfate monohydrate	0.1 M ADA pH 6.5	12% w/v Polyethylene glycol 4,000, 2% v/v 2-Propanol
24.	None	0.1 M ADA pH 6.5	1.0 M Ammonium phosphate dibasic
25.	0.1 M Magnesium chloride hexahydrate	0.1 M ADA pH 6.5	12% w/v Polyethylene glycol 6,000
26.	None	0.1 M ADA pH 6.5	12% v/v (+/-)-2-Methyl-2,4-pentanediol
27.	0.1 M Lithium sulfate monohydrate	0.1 M ADA pH 6.5	1.0 M Magnesium sulfate hydrate
28.	0.3 M Lithium sulfate monohydrate	0.1 M ADA pH 6.5	4% v/v Polyethylene glycol 400
29.	0.1 M Ammonium sulfate	0.1 M HEPES sodium pH 7.5	0.5 M Sodium phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic
30.	0.1 M Sodium chloride	0.1 M HEPES sodium pH 7.5	10% w/v Polyethylene glycol 4,000
31.	0.1 M Magnesium chloride hexahydrate	0.1 M HEPES sodium pH 7.5	18% v/v Polyethylene glycol 400
32.	None	0.1 M HEPES sodium pH 7.5	1.0 M Potassium sodium tartrate tetrahydrate
33.	0.1 M Ammonium sulfate	0.1 M HEPES sodium pH 7.5	18% v/v Polyethylene glycol 400
34.	0.1 M Ammonium sulfate	0.1 M HEPES sodium pH 7.5	10% w/v Polyethylene glycol 4,000
35.	0.1 M Sodium citrate tribasic dihydrate	0.1 M HEPES sodium pH 7.5	12% v/v (+/-)-2-Methyl-2,4-pentanediol
36.	None	0.1 M HEPES sodium pH 7.5	1.0 M Sodium citrate tribasic dihydrate
37.	0.6 M Magnesium sulfate hydrate	0.1 M HEPES sodium pH 7.5	4% v/v Polyethylene glycol 400
38.	0.6 M Magnesium sulfate hydrate	0.1 M HEPES sodium pH 7.5	4% v/v (+/-)-2-Methyl-2,4-pentanediol
39.	0.1 M Lithium sulfate monohydrate	0.1 M HEPES sodium pH 7.5	0.1 M Potassium sodium tartrate tetrahydrate
40.	0.1 M Lithium sulfate monohydrate	0.1 M Tris hydrochloride pH 8.5	12% v/v (+/-)-2-Methyl-2,4-pentanediol
41.	0.1 M Ammonium phosphate dibasic	0.1 M Tris hydrochloride pH 8.5	0.5 M Sodium phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic
42.	None	0.1 M Tris hydrochloride pH 8.5	0.1 M Sodium acetate trihydrate
43.	None	0.1 M Tris hydrochloride pH 8.5	0.1 M Sodium chloride
44.	0.1 M Ammonium phosphate dibasic	0.1 M Tris hydrochloride pH 8.5	12% w/v Polyethylene glycol 6,000
45.	0.1 M Potassium sodium tartrate tetrahydrate	0.1 M Tris hydrochloride pH 8.5	0.4 M Magnesium sulfate hydrate
46.	None	0.1 M Tris hydrochloride pH 8.5	0.2 M Lithium sulfate monohydrate
47.	None	0.1 M Tris hydrochloride pH 8.5	0.5 M Ammonium sulfate
48.	0.1 M Sodium citrate tribasic dihydrate	0.1 M Tris hydrochloride pH 8.5	5% v/v Polyethylene glycol 400

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

MembFac 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)

MembFac™ - HR2-114 Scoring Sheet	Date:	Date:	Date:
1. 0.1 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
2. 0.1 M Zinc acetate dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 12% w/v Polyethylene glycol 4,000			
3. 0.2 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6, 10% w/v Polyethylene glycol 4,000			
4. 0.1 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6, 12% v/v 2-Propanol			
5. 0.1 M Sodium acetate trihydrate pH 4.6, 12% w/v Polyethylene glycol 4,000			
6. 0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M Ammonium sulfate			
7. 0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M Magnesium sulfate heptahydrate			
8. 0.1 M Magnesium chloride hexahydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 18% v/v Polyethylene glycol 400			
9. 0.1 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M Ammonium phosphate monobasic			
10. 0.1 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6, 12% w/v Polyethylene glycol 6,000			
11. 0.1 M Magnesium chloride hexahydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 12% w/v Polyethylene glycol 6,000			
12. 0.1 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 18% v/v Polyethylene glycol 400			
13. 0.1 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 12 % w/v Polyethylene glycol 4,000			
14. 0.1 M Sodium citrate tribasic dihydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 10% v/v 2-Propanol			
15. 0.1 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
16. 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 1.0 M Magnesium sulfate heptahydrate			
17. 0.1 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 12% w/v Polyethylene glycol 4,000			
18. 0.1 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 12% w/v Polyethylene glycol 6,000			
19. 0.1 M Magnesium chloride hexahydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 4% v/v (+/-)-2-Methyl-2,4-pentanediol			
20. 0.1 M Sodium citrate trihydrate dihydrate pH 5.6, 0.1 M Sodium chloride			
21. 0.1 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 4% v/v Polyethylene glycol 400			
22. 0.1 M ADA pH 6.5, 1.0 M Ammonium sulfate			
23. 0.1 M Lithium sulfate monohydrate, 0.1 M ADA pH 6.5, 12% w/v Polyethylene glycol 4,000, 2% v/v 2-Propanol			
24. 0.1 M ADA pH 6.5, 1.0 M Ammonium phosphate dibasic			
25. 0.1 M Magnesium chloride hexahydrate, 0.1 M ADA pH 6.5, 12% w/v Polyethylene glycol 6,000			
26. 0.1 M ADA pH 6.5, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
27. 0.1 M Lithium sulfate monohydrate, 0.1 M ADA pH 6.5, 1.0 M Magnesium sulfate hydrate			
28. 0.3 M Lithium sulfate monohydrate, 0.1 M ADA pH 6.5, 4% v/v Polyethylene glycol 400			
29. 0.1 M Ammonium sulfate, 0.1 M HEPES sodium pH 7.5, 0.5 M Sodium phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic			
30. 0.1 M Sodium chloride, 0.1 M HEPES sodium pH 7.5, 10% w/v Polyethylene glycol 4,000			
31. 0.1 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 18% v/v Polyethylene glycol 400			
32. 0.1 M HEPES sodium pH 7.5, 1.0 M Potassium sodium tartrate tetrahydrate			
33. 0.1 M Ammonium sulfate, 0.1 M HEPES sodium pH 7.5, 18% v/v Polyethylene glycol 400			
34. 0.1 M Ammonium sulfate, 0.1 M HEPES sodium pH 7.5, 10% w/v Polyethylene glycol 4,000			
35. 0.1 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
36. 0.1 M HEPES sodium pH 7.5, 1.0 M Sodium citrate tribasic dihydrate			
37. 0.6 M Magnesium sulfate hydrate, 0.1 M HEPES sodium pH 7.5, 4% v/v Polyethylene glycol 400			
38. 0.6 M Magnesium sulfate hydrate, 0.1 M HEPES sodium pH 7.5, 4% v/v (+/-)-2-Methyl-2,4-pentanediol			
39. 0.1 M Lithium sulfate monohydrate, 0.1 M HEPES sodium pH 7.5, 0.1 M Potassium sodium tartrate tetrahydrate			
40. 0.1 M Lithium sulfate monohydrate, 0.1 M Tris hydrochloride pH 8.5, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
41. 0.1 M Ammonium phosphate dibasic, 0.1 M Tris hydrochloride pH 8.5, 0.5 M Sodium phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic			
42. 0.1 M Tris hydrochloride pH 8.5, 0.1 M Sodium acetate trihydrate			
43. 0.1 M Tris hydrochloride pH 8.5, 0.1 M Sodium chloride			
44. 0.1 M Ammonium phosphate dibasic, 0.1 M Tris hydrochloride pH 8.5, 12% w/v Polyethylene glycol 6,000			
45. 0.1 M Potassium sodium tartrate tetrahydrate, 0.1 M Tris hydrochloride pH 8.5, 0.4 M Magnesium sulfate hydrate			
46. 0.1 M Tris hydrochloride pH 8.5, 0.2 M Lithium sulfate monohydrate			
47. 0.1 M Tris hydrochloride pH 8.5, 0.5 M Ammonium sulfate			
48. 0.1 M Sodium citrate tribasic dihydrate, 0.1 M Tris hydrochloride pH 8.5, 5% v/v Polyethylene glycol 400			

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