MembFac™

Membrane Protein Crystallization Kit

コスモ・バイオ株式会社

HAMPTON RESEARCH

Solutions for Crystal Growth

User Guide **HR2-114**

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, Na Hepes, and Tris HCl 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.

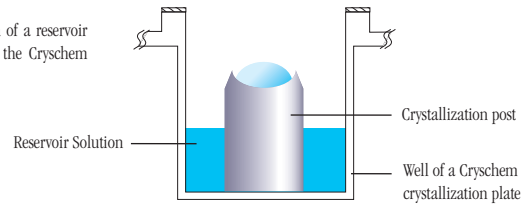
The sample should be as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation prior to use.

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.

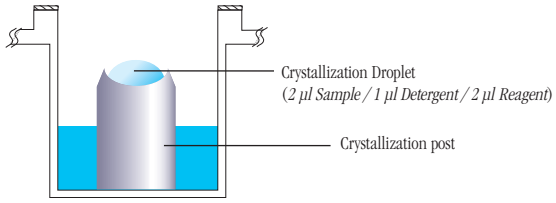


2. Pipet 2 µl of the sample to the center of the crystallization post of the Cryschem plate. See Figure 2.

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. After completing the twenty-four reservoirs seal the entire plate with two strips of Crystal Clear Sealing Tape (HR4-511).

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 72 crystallization detergents preformulated at 10 times the CMC for each detergent (Catalog Number: HR2-410, HR2-411, & HR2-412).

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 3 (on

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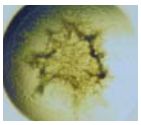
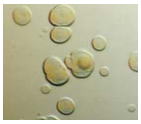
Figure 3
Typical observations in a crystallization experiment.


Clear Drop


Skin/Precipitate

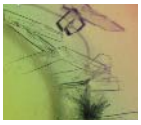

Precipitate


Precipitate/Phase

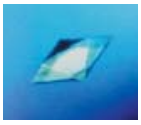

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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 CMCs, 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 heptane triol, 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 used before the "Best If Used By" date on the kit tubes. 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.

References and Readings

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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 5:00 p.m. USA Pacific Standard Time.

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