

## **Crystallization Basic Kit for Membrane Proteins**

Product Number **73513**

Store at 4°C

### **TECHNICAL BULLETIN**

#### **Application**

Crystallization Basic Kit for Membrane Proteins is a rapid empirical screening method to determine the best conditions for the crystallization of membrane proteins and biological macromolecules. Crystallization Basic Kit for Membrane Proteins is also effective in determining the solubility of a macromolecule in a wide range of precipitants and pH.

Membrane proteins are often difficult to crystallize. The hydrophobic nature of these proteins leads to amorphous aggregation in aqueous solutions, rather than crystal formation. These proteins are routinely purified with a detergent, which provides the best protein stability and activity in solution. The presence of detergents increases the number of variables critical to crystallization for membrane proteins compared to soluble proteins. The reagents in this kit are specifically formulated for screening of crystallization conditions in conjunction with the screening of crystallization detergents.

The reagents of this kit vary in pH, along with changes in the concentration and content of buffer, salt, and precipitant. The buffers, sodium acetate, sodium citrate, ADA, HEPES sodium salt, and Tris-HCl, allow testing at five different pH values, 4.6, 5.6, 6.5, 7.5, and 8.5, respectively. Individual volatile agents, non-volatile agents, and salts, along with combinations of these three, comprise the four groups of precipitating agents.

#### **Crystallization Basic Kit for Membrane Proteins Formulation/Storage/Stability**

Crystallization Basic Kit for Membrane Proteins reagents are formulated using high purity reagents (mostly Microselect from Fluka) and ultrapure water. There are many experiences where the Microselect chemicals have successfully been used for different crystallization methods. All solutions are sterile filtered using 0.22 micron filters.

All solutions are available separately as 100 ml bottles.

Crystallization Basic Kit for Membrane Proteins reagents are stable at room temperature, if the bottles aren't opened. To enhance reagent stability, it is strongly recommended that kit reagents be stored at 4°C or -20°C. Don't set the reagents under ultraviolet light to protect them from microorganisms.

If the sample contains phosphate, borate, or carbonate buffers, it is possible to obtain inorganic crystals when using those 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 buffers with a more soluble buffer that does not complex with divalent cations.

#### **Sample Preparation**

The macromolecular membrane protein 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.

A sample concentration of 10 to 20 mg/ml in water is recommended and the detergent concentration should only be slightly above the critical micelle concentration (CMC).

#### **Procedures**

The following procedure describes the use of Crystallization Basic Kit for Membrane Proteins with the Sitting Drop Vapor Diffusion method. It's also usual to use the Hanging Drop method for the Crystallization Kit for Membrane Proteins. Directions for the Hanging, Sitting Drop and other crystallization methods are available from Fluka Techservice.

Prepare a Cryschem Plate (from Emerald BioStructures) or MVD/24 plate (from Charles Supper Company) for Sitting Drop Vapor Diffusion. If of using cover slides it is recommended that on each upper edge of the 24 wells should be put a thin film of grease. This ensures that the cover slide doesn't shift. There is no need for cover slides if sealing tape is used. 48 wells are to be prepared for a complete Crystallization Basic Kit for Membrane Proteins. See figure 1 and 2 next page.

1. Using a clean pipet tip for each reagent, pipet 800  $\mu$ l of Crystallization Basic Kit for Membrane Proteins reagent 1 into the first well, A1. Repeat the procedure for the remaining reagents using a clean pipet tip for each reagent.



Figure 1

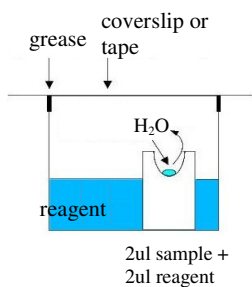


Figure 2

2. Pipet 2  $\mu$ l of the sample in to the dents of the crystallization post of the plate. See figure 2 above.
3. It's possible to pipet the detergent directly into the sample, or dilute the detergent in the reservoir and then pipet the solution of the reservoir into the drop of the sample. Because of the prior equilibration with the reservoir, the crystallization screening detergent concentration in the drop should

be 1 to 3 times the critical micelle concentration. When the detergent is directly placed into the drop, pipet 1  $\mu$ l of the corresponding crystallization screening detergent (suggested stock detergent concentration equal to ten times the critical micelle concentration) into the 2  $\mu$ l sample drop.

4. Pipet 2  $\mu$ l of Crystallization Basic Kit for Membrane Proteins reagent 1 from well A1 into the sample droplet. Mix by aspirating and dispensing the droplet with the pipet. Keep the tip in the drop during mixing to avoid foaming.
5. Repeat 3. and 4. using the remaining reagents.
6. Either seal the entire plate with clear sealing tape (Stratech, Molecular Dimension Ltd) or seal the individual wells with cover slides and sealants.
7. The Crystallization Basic Kit for Membrane Proteins can be performed in duplicate at 4 °C and at room temperature if sample quantities permit. Incubate and store the plates in a place with stable temperature and free of vibration.

## Detergent Considerations

The Crystallization Basic Kit for Membrane Proteins allows for sparse matrix sampling for screening of crystallization reagents with a given crystallization detergent. Important properties to be considered during the selection of detergents for use in crystallization screening are the CMC, micelle size, molecular weight, and the hydrophobic tail of the detergent molecule, which binds to the hydrophobic regions of the protein. These properties can change several parameters (protein-protein, protein-detergent, protein-solvent, and detergent-detergent interactions) of the sample liquid dynamics.

The detergent dimension in solution (micelle size) is a parameter critical in membrane protein crystallization. It is recommended to start screening with detergents, which have the smallest micelle sizes and progress to those with larger micelle sizes. A screen of the reagents in this kit should be performed for each

detergent screened. For example, the following order would be suggested:

n-hexyl- $\beta$ -D-glucoside (Fluka 53180)  
Zwittergent 3-10 (Fluka 30694)  
n-octyl- $\beta$ -D-glycoside(Fluka 75083)  
nonyl- $\beta$ -D-glycoside (Fluka 74420)  
LDAO (Fluka 40234),  
CYMOL<sup>®</sup>-6 (Fluka 29396),  
C<sub>12</sub> E<sub>8</sub> (Fluka 74680).

Another concern in crystallizing membrane proteins is phase separation caused by the presence of detergents in salt solutions. The presence of salts may raise the ionic strength of the solution to a point where the detergent will partition into a separate phase. The hydrophobic protein will migrate into the detergent rich phase and denature. The use of amphiphiles like in table 1 (0.5 to 1.0%) may help prevent phase separation:

product	cmc [mM]	MW [g/mol]	density [kg/l]
1,2,3 heptanetriol (Fluka 51845, 51846)		148.2	solid
2-methyl-2,4-pentandiol (Fluka 68338)		118.18	0.922
benzamidine HCl (Fluka 12073)		156.62	solid
hexyl-beta-D-glucopyranoside (Fluka 53180)	250	264.32	solid
1,6 hexanediol (Fluka 52800)		118.18	solid
nonyl-beta-D-glucopyranoside (Fluka 74420)	6.5	306.4	solid
6-cyclohexylhexyl-beta-D-maltoside (Fluka 29396)	0.56	508.61	solid
n-octyl-beta-D-glucoside (Fluka 75083)	80	292.38	solid
ethylene glycol (Fluka 03747)		62.07	1.113
Zwittergent 3-10 (Fluka 30694)	25-40	307.49	solid
1,2 dimethoxyethane (Fluka 38569)		90.12	0.87
CTAB (Fluka 52365).	1	364.46	solid

table 1 with amphiphiles their critical micelle concentration (cmc) molecular weight and density. (out of literature)

The use of crystallization detergents is not entirely harmless in all cases. At too high a concentration, they can denature the target protein. Myoglobin can be denatured by detergents, either by solubilizing the heme pocket or by solubilizing the heme itself.

### Examine The Drop

Immediately after the screen is set up, examine the droplets under a stereomicroscope at 10 to 100X magnification. Scan the focal plane for small crystals and record observations for all droplets. For the first week, information should be recorded daily and, thereafter, on a weekly basis. Records should include the clarity of the droplet (clear, precipitate, or crystals), along descriptive phrases and a numerical scale. The following are possible examples:

8(= single crystals) small tetrahedral,  
5(=Possetes of Sperulites) red/brown,  
1(=clear drop), green  
6(= needle shaped crystals) in a fine yellow precipitate.

It is useful to record the largest crystal size.

### Interpreting Crystallization Kit for Membrane Proteins

A clear drop may be an indication that the drop has not yet reached complete equilibration. If the drop remains clear after 3 to 4 weeks, the relative sample and reagent supersaturation may be too low. One may repeat the screen using a protein sample at twice the initial concentration. If more than two thirds of the drops remain clear, consider repeating the entire screen using a 2-fold concentrated protein sample.

A drop containing precipitate indicates that the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. In this case, reduce the biomolecule concentration and repeat the test. If more than two thirds of the drops contain precipitate and no crystals are present, consider diluting the sample concentration to half of the concentration 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). Sample purity may also cause precipitation. Low sample purity, aggregation, or a heterogeneous preparation may be responsible for precipitation. In these cases, further sample

purification is required. Do not discard or ignore a drop containing a precipitate as it is possible for crystals to form from a precipitate. If possible, use a high power microscope to examine the precipitate between crossed polarizing lenses. True amorphous precipitates do not glow.

Birefringence microcrystalline precipitates can glow as a result of the plane of polarization. It may be possible to use streak seeding to produce larger crystals from microcrystalline precipitates.

If macromolecular crystals are obtained during an initial screen, the conditions may be optimized by varying the pH, salt type, salt concentration, precipitant type, precipitant concentration, sample concentration, temperature, additives, and other crystallization variables.

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.

Store the plates until the drops are dried out. Crystal can grow really fast (in 15 minutes) or may need more time (up to 1 year).

## References

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