

MemSys™ MD1-25

The original systematic screen for membrane proteins.

48 conditions allowing the pH range, precipitants and salts used in membrane protein crystallization to be screened with detergent containing protein drops. The reagents can be easily arranged in systematic array to facilitate the interpretation of results and the design of further optimization experiments.

MD1-25 is presented as 48 x 10 mL conditions.

Features of MemSys™:

- Ideal for initial crystallisation screening of membrane proteins.
- Membrane protein solubility is pushed to the limit to provide more information than previous sparse matrix type screens
- Includes the pH, precipitant concentration and type, and salts found to be successful.
- Primarily designed for alpha type transmembrane proteins, but also been successfully applied to beta type outer membrane proteins.

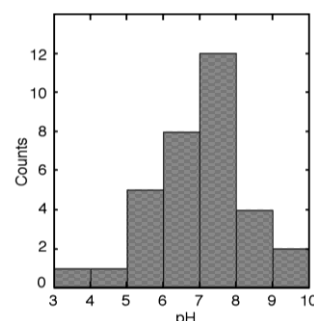
Introduction

MemSys™ offers a systematic approach to screening for initial crystallization conditions for membrane proteins using vapour diffusion methods. The systematic approach where hopefully membrane protein solubility is pushed to the limit aims to provide more information than previous sparse matrix type screens. Whilst primarily designed for alpha type transmembrane proteins this screen has also been successfully applied to beta type outer membrane proteins (unpublished results) and is expected to be equally applicable to all membrane protein types.

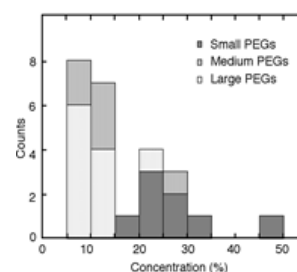
Recently there has been an important increase in the number of membrane protein structures solved providing a much larger database of reported conditions for successful crystallization.

MemSys™ is a systematic screen spanning the key values of pH, precipitant type/concentration, and

salts ("Methods and Results in the Crystallization of Membrane Proteins" Ed. Iwata S. International University Line). MemSys™ contains 48 conditions allowing the pH range, precipitants and salts used in membrane protein crystallization to be screened with detergent containing protein drops. The reagents can be easily arranged in a systematic array to facilitate the interpretation of results and the design of further optimization experiments.



Typical pH conditions used for membrane protein crystallization.



Types and concentrations of PEGs used for membrane protein crystallization.

(Small PEGs include triethylene glycol, PEG400 and PEG550 monomethylether. Medium PEGs include PEG1500, PEG2000 and PEG2000 monomethylether. Large PEGs include PEG3350, PEG4000, PEG6000 and PEG10000.)

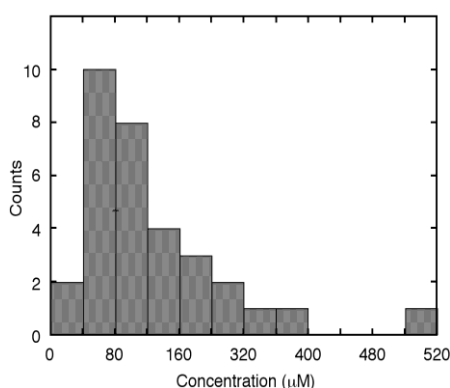
Instructions for Use

MemSys™ is intended to be used in vapour diffusion crystallization methods. The protein drop is normally diluted 1:1 with the screening reagent. Detergents should also be added to this drop.

Membrane protein sample preparation

Membrane proteins often form aggregates and these will not crystallize. Electron microscopy and analytical ultracentrifugation can be more appropriate than dynamic light scattering for assessing sample homogeneity/ monodispersity of membrane protein samples prior to setting up crystallization experiments. Sample monodispersity can be improved by changing the detergent, increasing salt concentration, and ultracentrifugation.

Typical protein concentrations for crystallizing membrane proteins are in the range 40 - 80 μM . A good starting point would be 50 μM (10 mg/mL for a 200 kDa protein). Protein concentrations for crystallizing membrane proteins tend to be somewhat higher than normally recommended for soluble proteins, so if 50 μM is not successful try 100 μM (or even higher, it is often easier than changing the precipitant concentration).

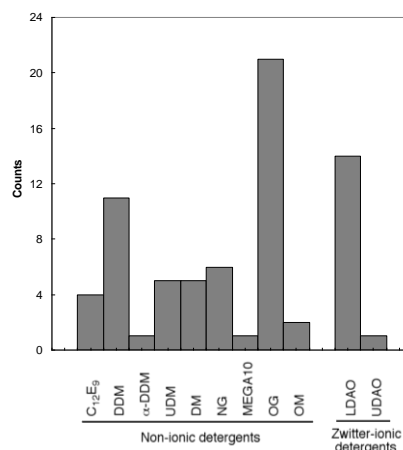


Typical protein concentrations used for membrane protein crystallization.

Detergents

Often the choices of detergent or precise concentration are critical parameters for initial screening. Good starting detergents are N-octyl β -D-Octyl glucopyranoside (OG), N-dodecyl β -D-maltoside (DDM) or N,N-dimethyldodecylamine N-oxide (LDAO). It is worth trying to crystallize with the detergent that was used during purification. Typically a concentration

around 2 - 3 times the critical micelle concentration (CMC) should be used.



Detergents used for membrane protein crystallisation.

C₁₂E₉ (dodecyl nonaoxyethylene ether), *DDM* (N-dodecyl β -D-maltoside), α -*DDM* (N-dodecyl α -D-maltoside), *UDM* (N-undecyl β -D-maltoside), *DM* (N-decyl β -D-maltoside), *NG* (N-nonyl β -D-glucopyranoside), *MEGA10* (N-decanoyl-N-methylglucamin), *OG* (N-octyl β -D-Octyl glucopyranoside), *OM* (octyl- β -D-maltoside), *LDAO* (N,N-dimethyldodecylamine N-oxide), *UDAO* (N,N-dimethylundecylamine N-oxide).

Once a result is obtained, optimization of detergent choice and concentration is critical to obtain good quality crystals and a second detergent is often used as an additive (see below).

pH

The pH of the protein drop should not be overlooked. Most of the kit reagents are buffered and to take full advantage of this, a low concentration (20 mM) of buffer in the protein sample is desirable. Ionic strength can be increased with sodium chloride (50 - 100 mM) if protein solubility becomes a problem.

Additives

The use of additives in the protein drop has often been found useful, or even essential, for optimizing the crystal quality of membrane proteins. Whilst additives are normally added to the protein drop, volatile additives must also be included in the well (reservoir) solution. 1,2,3-heptanetriol (1 - 6 %) has been the most successfully used additive. Other additives often used are: benzamidine (2 - 4 %), glycerol (10 - 20 %), ethanol (5 - 10 %) and DMSO (5 -

10 %). As mentioned above, second detergents are also often used as additives to optimize crystal quality.

Temperature

Temperature is a critical parameter for crystallization due to the temperature dependence of solubility. Membrane protein crystals are often temperature sensitive and so crystallization experiments should be observed at the temperature at which they have been purified. Crystallization screens should be performed at multiple temperatures (e.g. 4°C and 21°C) if sample quantities permit.

Observation of results

Under optimized conditions crystals can grow quite quickly. A useful regime is to check for crystal growth at 1, 3, 7, 14 and 30 days. MemSys™ reagents are numbered according to precipitant and pH to facilitate analysis of screening results, and to plan optimization experiments.

Formulation notes

MemSys™ reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Final pH may vary from that specified on the datasheet

Contact Us

Individual reagents, detergents and stock solutions for optimization are available from Molecular Dimensions.

Enquiries regarding MemSys™ formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at

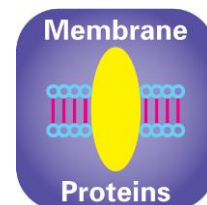
www.moleculardimensions.com.

References

Methods and Results in Crystallization of Membrane Proteins. (2003), IUL Biotechnology Series, **4**. Ed. Iwata S. ISBN: 0-9636817-9-6.

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Molecular Dimensions acknowledges the work of Prof S Iwata, Dr M Iwata and Dr J Abramson in designing this product.



MemSys™

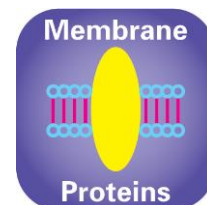
Conditions 1 -48

MD1-25

Tube #	Conc.	Salt1	Conc.	Salt2	Conc.	Buffer	pH	Conc.	Precipitant
1	2.5 M	Ammonium sulfate			0.1 M	Sodium citrate	5.5		
2	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium citrate	3.5	30 % v/v	PEG 400
3	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate	4.5	30 % v/v	PEG 400
4	0.1 M	Sodium chloride			0.1 M	Sodium citrate	5.5	30 % v/v	PEG 400
5	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium citrate	5.5	30 % v/v	PEG 400
6	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium citrate	5.5	30 % v/v	PEG 400
7	2.5 M	Ammonium sulfate			0.1 M	MES	6.5		
8					0.1 M	MES	6.5	30 % v/v	PEG 400
9	0.1 M	Sodium chloride			0.1 M	MES	6.5	30 % v/v	PEG 400
10	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	MES	6.5	30 % v/v	PEG 400
11	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	MES	6.5	30 % v/v	PEG 400
12					0.1 M	MOPS	7.0	30 % v/v	PEG 400
13	2.5 M	Ammonium sulfate			0.1 M	Sodium HEPES	7.5		
14	0.1 M	Sodium chloride			0.1 M	MOPS	7.0	30 % v/v	PEG 400
15					0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
16	0.1 M	Sodium chloride			0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
17	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
18	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
19	1.5 M	Lithium sulfate			0.1 M	Tris	8.5		
20	0.1 M	Sodium chloride			0.1 M	Tris	8.5	30 % v/v	PEG 400
21	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Tris	8.5	30 % v/v	PEG 400
22	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	30 % v/v	PEG 400
23	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	CAPSO	9.5	30 % v/v	PEG 400
24	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	CAPSO	9.5	30 % v/v	PEG 400
25	1.5 M	Sodium phosphate monobasic monohydrate			0.1 M	Sodium citrate	5.5		
26	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium citrate	3.5	12 % w/v	PEG 4000
27	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium acetate	4.5	12 % w/v	PEG 4000
28	0.1 M	Sodium chloride			0.1 M	Sodium citrate	5.5	12 % w/v	PEG 4000
29	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium citrate	5.5	12 % w/v	PEG 4000
30	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium citrate	5.5	12 % w/v	PEG 4000
31	1.5 M	Sodium phosphate monobasic monohydrate			0.1 M	MES	6.5		
32					0.1 M	MES	6.5	12 % w/v	PEG 4000
33	0.1 M	Sodium chloride			0.1 M	MES	6.5	12 % w/v	PEG 4000
34	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	MES	6.5	12 % w/v	PEG 4000
35	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	MES	6.5	12 % w/v	PEG 4000
36					0.1 M	MOPS	7.0	12 % w/v	PEG 4000
37	1.5 M	Potassium phosphate dibasic			0.1 M	Sodium HEPES	7.5		
38	0.1 M	Sodium chloride			0.1 M	MOPS	7.0	12 % w/v	PEG 4000
39					0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
40	0.1 M	Sodium chloride			0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
41	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
42	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
43	1.5 M	Potassium phosphate dibasic			0.1 M	Tris	8.5		
44	0.1 M	Sodium chloride			0.1 M	Tris	8.5	12 % w/v	PEG 4000
45	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Tris	8.5	12 % w/v	PEG 4000
46	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	12 % w/v	PEG 4000
47	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	CAPSO	9.5	12 % w/v	PEG 4000
48	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	CAPSO	9.5	12 % w/v	PEG 4000

Abbreviations:

CAPSO; 3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic Acid Sodium Salt, **Sodium HEPES**; N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid sodium salt, **MES**; 2-(N-morpholino)ethanesulfonic acid, **MOPS**; 3-(N-Morpholino)-propanesulfonic acid, **PEG**; Polyethylene glycol, **Tris**; 2-Amino-2-(hydroxymethyl)propane-1,3-diol.



Manufacturer's safety data sheets are available from our website or by scanning the QR code here:



Re-Ordering details:

Catalogue Description	Pack size	Catalogue Code
MemSys™	48 x 10 mL	MD1-25
MemStart™ + MemSys™ HT-96	96 x 1 mL	MD1-33
The Membrane Protein Combination (MemStart™ + MemSys™)	96 x 10ml	MD1-04
Single Reagents		
MemSys™ single reagents	100 mL	MDSR-25-tube number
MemStart™ + MemSys™ HT-96 single reagents	100 mL	MDSR-33-well number

For MemSys™ stock solutions please visit the Optimization section on our website.