MemStart™ MD1-21

A starting point for screening and optimizing crystallization conditions for alpha - helical type transmembrane proteins using vapour diffusion methods.

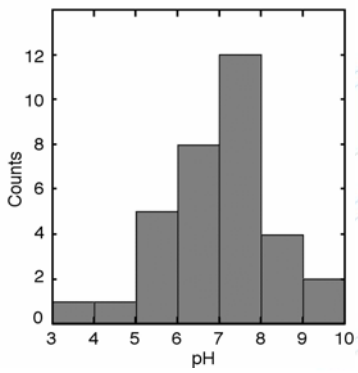
A targeted sparse matrix of 48 conditions (10 ml) allowing the pH range, precipitants and salts used in membrane protein crystallization to be screened with detergent- containing protein drop

Features of MemStart:

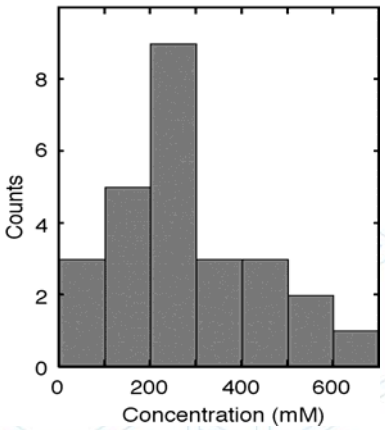
- Based on the reagents typically used in the laboratory of Prof. S. Iwata.
- Optimized to span 33 reported successful crystallization conditions for which high resolution structures of membrane proteins have been determined, including pH, type of precipitant, precipitant concentration, and salts.

Introduction

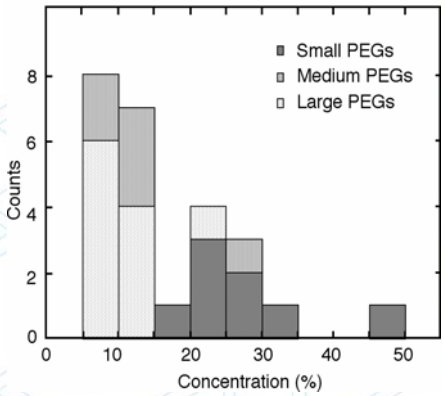
This kit is intended as a starting point for screening and optimizing crystallization conditions for alpha – helical type transmembrane proteins using vapour diffusion methods. Recently, there has been an increase in the number of membrane protein structures solved, providing a much larger database of reported conditions for successful crystallization. This kit is based on the reagents typically used in the laboratory of Prof. S. Iwata at Imperial College, London and is optimized to span the 33 reported successful crystallization conditions of membrane proteins for which high resolution structures have been determined.



Typical pH conditions used for membrane protein crystallization.



Total concentration of salts 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

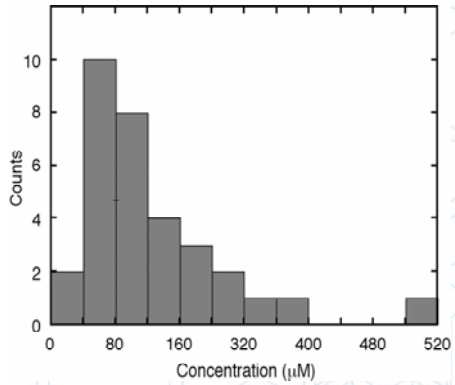
MemStart is intended to be used in vapour diffusion crystallization methods. The protein drop is normally diluted 1:1 with the screening reagent. Detergents should also be added to this drop.

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.

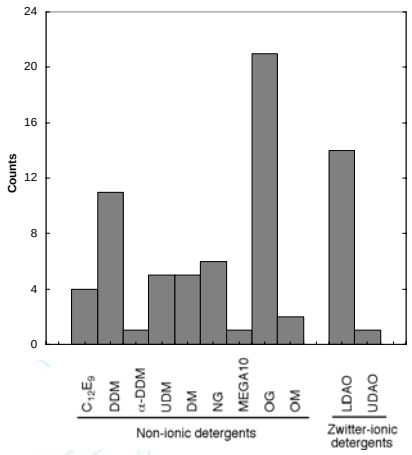
Typical protein concentrations for crystallizing membrane proteins are in the range 40 - 80 µM. A good starting point would be 50 µM (10 mg/ml for a 200 kDa protein). Protein concentrations for crystallizing membrane proteins tend to be somewhat higher than normally recommended for soluble proteins, 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.

$C_{12}E_9$ (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-ß-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. MemStart reagents are numbered according to precipitant and pH to facilitate analysis of screening results, and to plan optimization experiments.

Formulation notes

MemStart 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 MemStart 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.

This product is manufactured under an exclusive licence from Imperial College of Science, Technology & Medicine, London, UK.
Molecular Dimensions acknowledges the work of Prof S Iwata, Dr M Iwata and Dr J Abramson in designing this product.

References

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

MemStart™ MD1-21

Tube #	Salt	Buffer	pH	Precipitant
1	None	0.1 M sodium acetate	4.6	2 M ammonium sulfate
2	None	0.1 M ADA	6.5	1 M ammonium sulfate
3	None	None	-	2 M ammonium sulfate
4	None	0.1 M Tris	8.5	2 M ammonium sulfate
5	None	0.1 M Na HEPES	7.5	1.5 M lithium sulfate
6	None	0.1 M sodium acetate	4.6	1 M magnesium sulfate
7	None	0.1 M tri-sodium citrate	5.6	1 M magnesium sulfate
8	0.1 M lithium sulfate	0.1 M ADA	6.5	1 M magnesium sulfate
9	None	0.1 M ammonium dihydrogen phosphate	6.5	None
10	0.1 M ammonium sulfate	0.5 M di-potassium hydrogen phosphate/ 0.5 M di-sodium hydrogen phosphate	7.5	None
11	0.1M lithium sulfate	0.1 M sodium acetate	4.6	1 M ammonium dihydrogen phosphate
12	None	0.1 M tri-sodium citrate	5.6	1 M ammonium dihydrogen phosphate
13	None	0.1 M Tris	8.5	2 M ammonium dihydrogen phosphate
14	None	None	4.6	2 M sodium formate
15	None	None	-	4 M sodium formate
16	None	0.1 M MES	6.5	1.4 M sodium acetate
17	None	0.1 M Na HEPES	7.5	1.4 M tri-sodium citrate
18	None	0.1 M Na HEPES	7.5	1 M potassium sodium tartrate
19	None	0.1 M Na HEPES	7.5	2 % v/v PEG 400/ 2 M ammonium sulfate
20	0.1M magnesium chloride	0.1 M sodium acetate	4.6	30 % v/v PEG 400
21	0.1M sodium chloride	0.1 M tri-sodium citrate	5.6	30 % v/v PEG 400
22	0.1M lithium sulfate	0.1 M tri-sodium citrate	5.6	30 % v/v PEG 400
23	0.3 M lithium sulfate	0.1 M ADA	6.5	30 % v/v PEG 400
24	0.1 M magnesium chloride	0.1 M Na HEPES	7.5	30 % v/v PEG 400
25	0.1 M ammonium sulfate	0.1 M Na HEPES	7.5	30 % v/v PEG 400
26	0.2 M tri-sodium citrate	0.1 M Tris	8.5	30 % v/v PEG 400
27	0.1 M zinc acetate	0.1 M sodium acetate	4.6	12 % w/v PEG 4K
28	0.2 M ammonium sulfate	0.1 M sodium acetate	4.6	12 % w/v PEG 4K
29	None	0.1 M sodium acetate	4.6	12 % w/v PEG 4K
30	0.1 M lithium sulfate	0.1 M tri-sodium citrate	5.6	12 % w/v PEG 4K
31	0.1 M sodium chloride	0.1 M tri-sodium citrate	5.6	12 % w/v PEG 4K
32	0.1 M lithium sulfate	0.1 M ADA	6.5	12 % w/v PEG 4K
33	0.1 M sodium chloride	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
34	0.1 M ammonium sulfate	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
35	0.2 M magnesium chloride	0.1 M Tris	8.5	12 % w/v PEG 4K
36	0.2 M lithium sulfate hydrate	0.1 M Tris	8.5	12 % w/v PEG 4K
37	0.2 M ammonium sulfate	None	-	12 % w/v PEG 4K
38	0.1 M sodium chloride	0.1 M sodium acetate	4.6	12 % w/v PEG 6K
39	0.1 M magnesium chloride	0.1 M sodium acetate	4.6	12 % w/v PEG 6K
40	0.1 M magnesium chloride	0.1 M ADA	6.5	12 % w/v PEG 6K
41	0.1 M di-ammonium hydrogen phosphate	0.1 M Tris	8.5	12 % w/v PEG 6K
42	1 M lithium sulfate	None	-	2 % w/v PEG 8K
43	0.2 M sodium acetate	0.1 M MES	6.5	10 % w/v PEG 8K
44	0.2 M zinc acetate	0.1 M MES	6.5	10 % w/v PEG 8K
45	0.2 M calcium acetate	0.1 M MES	6.5	10 % w/v PEG 8K
46	None	0.1 M Tris	8.5	10 % w/v PEG 8K
47	0.2 M ammonium sulfate	None	-	10 % w/v PEG 8K
48	0.5 M lithium sulfate	None	-	10 % w/v PEG 8K

Abbreviations:
ADA; N-(2-Acetamido)iminodiacetic Acid, **HEPES**; N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid, **MES**; 2-(N-morpholino)ethanesulfonic acid, **MME**; Monomethylether, **PEG**; Polyethylene glycol (4K, 6K and 8K correspond to the molecular weight, in thousands of Daltons, of PEG), **Tris**; 2-Amino-2-(hydroxymethyl)propane-1,3-diol.

Note: The pH of each final reagent is checked and adjusted back to the stated pH of the buffer (±0.2 pH units) as appropriate.

Manufacturer's datasheets are available on request

moleculardimensions.com

Grid

MemSys™ MD1-25

A systematic screen spanning the key values of pH, precipitant type/concentration, and salts.

48 conditions (10 ml) 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.

Features of MemSys:

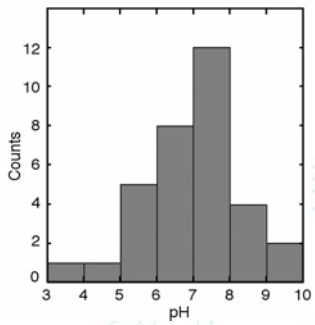
- A systematic approach to screening for initial crystallisation conditions for membrane proteins using vapour diffusion methods.
- Membrane protein solubility is pushed to the limit to provide more information than previous sparse matrix type screens.
- Includes the pH, precipitant concentration and 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

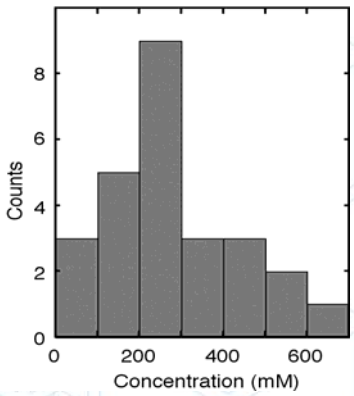
MemSys offers a systematic approach to screening for initial crystallization conditions for membrane proteins using vapour diffusion methods. The systematic approach where hopefully membrane protein solubility is pushed to the limit aims to provide more information than previous sparse matrix type screens. 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.

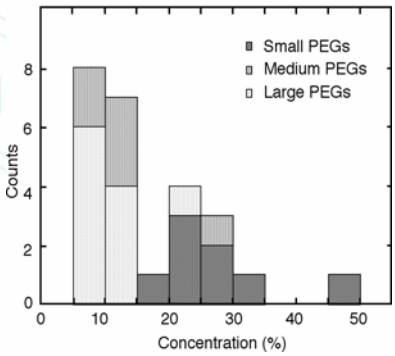
MemSys is a systematic screen spanning the key values of pH, precipitant type/concentration, and salts ("Methods and Results in the Crystallization of Membrane Proteins" Ed. Iwata S. In Press, International University Line). MemSys contains 48 conditions allowing the pH range, precipitants and salts 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.



Total concentration of salts 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

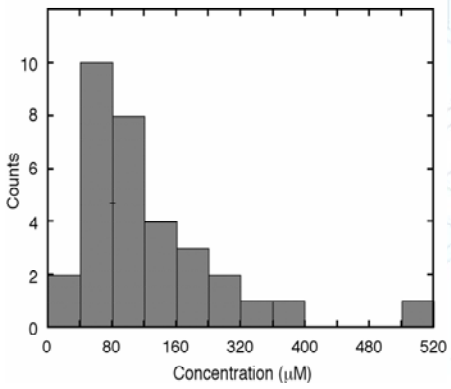
MemSys is intended to be used in sitting drop or hanging drop vapour diffusion crystallization methods. The protein drop will normally be diluted 1+1 with the screening reagent and the drop will of course also contain detergent.

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).

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.



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.

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

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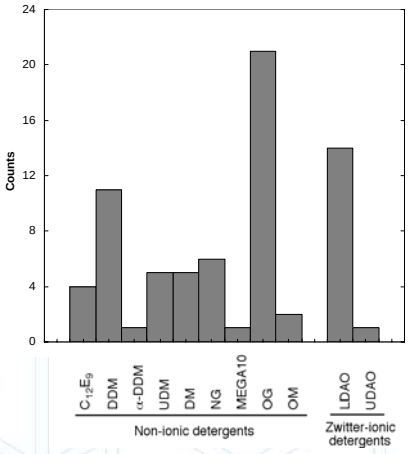

Detergents used for membrane protein crystallisation.

$C_{12}E_9$ (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-ß-D-maltoside), LDAO (N,N-dimethyldodecylamine N-oxide), UDAO (N,N-dimethylundecylamine N-oxide).

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.

This product is manufactured under an exclusive licence from Imperial College of Science, Technology & Medicine, London, UK.

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

References

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

MemSys MD1-25

Tube No.	Salt 1	Salt 2	Buffer	pH	Precipitant
1.	None	None	0.1 M Na citrate	5.5	2.5 M ammonium sulfate
2.	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na citrate	3.5	30 % v/v PEG 400
3.	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na acetate	4.5	30 % v/v PEG 400
4.	0.1 M sodium chloride	None	0.1 M Na citrate	5.5	30 % v/v PEG 400
5.	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na citrate	5.5	30 % v/v PEG 400
6.	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na citrate	5.5	30 % v/v PEG 400
7.	None	None	0.1 M MES	6.5	2.5 M ammonium sulfate
8.	None	None	0.1 M MES	6.5	30 % v/v PEG 400
9.	0.1 M sodium chloride	None	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	0.1 M MES	6.5	30 % v/v PEG 400
12.	None	None	0.1 M MOPS	7.0	30 % v/v PEG 400
13.	None	None	0.1 M Na HEPES	7.5	2.5 M ammonium sulfate
14.	0.1 M sodium chloride	None	0.1 M MOPS	7.0	30 % v/v PEG 400
15.	None	None	0.1 M Na HEPES	7.5	30 % v/v PEG 400
16.	0.1 M sodium chloride	None	0.1 M Na HEPES	7.5	30 % v/v PEG 400
17.	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na HEPES	7.5	30 % v/v PEG 400
18.	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na HEPES	7.5	30 % v/v PEG 400
19.	None	None	0.1 M Tris	8.5	1.5 M lithium sulfate
20.	0.1 M sodium chloride	None	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	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	0.1 M CAPSO	9.5	30 % v/v PEG 400
25.	None	None	0.1 M Na citrate	5.5	1.5 M sodium phosphate
26.	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na citrate	3.5	12 % w/v PEG 4K
27.	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na acetate	4.5	12 % w/v PEG 4K
28.	0.1 M sodium chloride	None	0.1 M Na citrate	5.5	12 % w/v PEG 4K
29.	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na citrate	5.5	12 % w/v PEG 4K
30.	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na citrate	5.5	12 % w/v PEG 4K
31.	None	None	0.1 M MES	6.5	1.5 M sodium phosphate
32.	None	None	0.1 M MES	6.5	12 % w/v PEG 4K
33.	0.1 M sodium chloride	None	0.1 M MES	6.5	12 % w/v PEG 4K
34.	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M MES	6.5	12 % w/v PEG 4K
35.	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M MES	6.5	12 % w/v PEG 4K
36.	None	None	0.1 M MOPS	7.0	12 % w/v PEG 4K
37.	None	None	0.1 M Na HEPES	7.5	1.5 M potassium phosphate
38.	0.1 M sodium chloride	None	0.1 M MOPS	7.0	12 % w/v PEG 4K
39.	None	None	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
40.	0.1 M sodium chloride	None	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
41.	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
42.	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
43.	None	None	0.1 M Tris	8.5	1.5 M potassium phosphate
44.	0.1 M sodium chloride	None	0.1 M Tris	8.5	12 % w/v PEG 4K
45.	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Tris	8.5	12 % w/v PEG 4K
46.	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Tris	8.5	12 % w/v PEG 4K
47.	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M CAPSO	9.5	12 % w/v PEG 4K
48.	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M CAPSO	9.5	12 % w/v PEG 4K

Abbreviations:
CAPSO; 3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic Acid Sodium Salt, **Na 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 (4K correspondS to the molecular weight, in thousands of Daltons, of PEG), **Tris**; 2-Amino-2-(hydroxymethyl)propane-1,3-diol.

Manufacturer's safety data sheets are available upon request.