Molecular Dimensions

CryoProtX™ MD1–61

"Get better results from your crystals"

This kit is designed for the cryoprotection of protein crystals and is for use after the crystals have been grown. Get the most out of your protein crystals with this easy-to-use pre-mixed cryo-kit.

MD1-61 is presented as a 46 x 1.5 mL kit.

To be used in conjunction with the Quick-Start Guide.

Features of CryoProtX™:

- Preserve the highest diffraction potential of crystals.
- Ready-made mixtures.
- Incorporates pH screening.
- Crystals less likely to crack or dissolve.
- Ideal for heavy atom, ligand or back soaks.
- Screen with low-affinity, low-solubility inhibitors.
- Customizable to suit specific projects.

CryoProtX™ has been developed to aid the crystallographer in finding the best cryoprotectant for their protein crystals.

It allows you to test mixed formulations for cryo-protection in a very simple and easy-to-use way. Mixes of cryoprotectants (CryoMixes™) Figure 1 are provided ready-to-use for single-step cryosoaking (See Quick-Start Guide). Further components of salts, PEG and sugar groups are provided for fine-tuning of your cryoprotectant mix and to improve upon the cryoprotectants based on previous crystal projects of

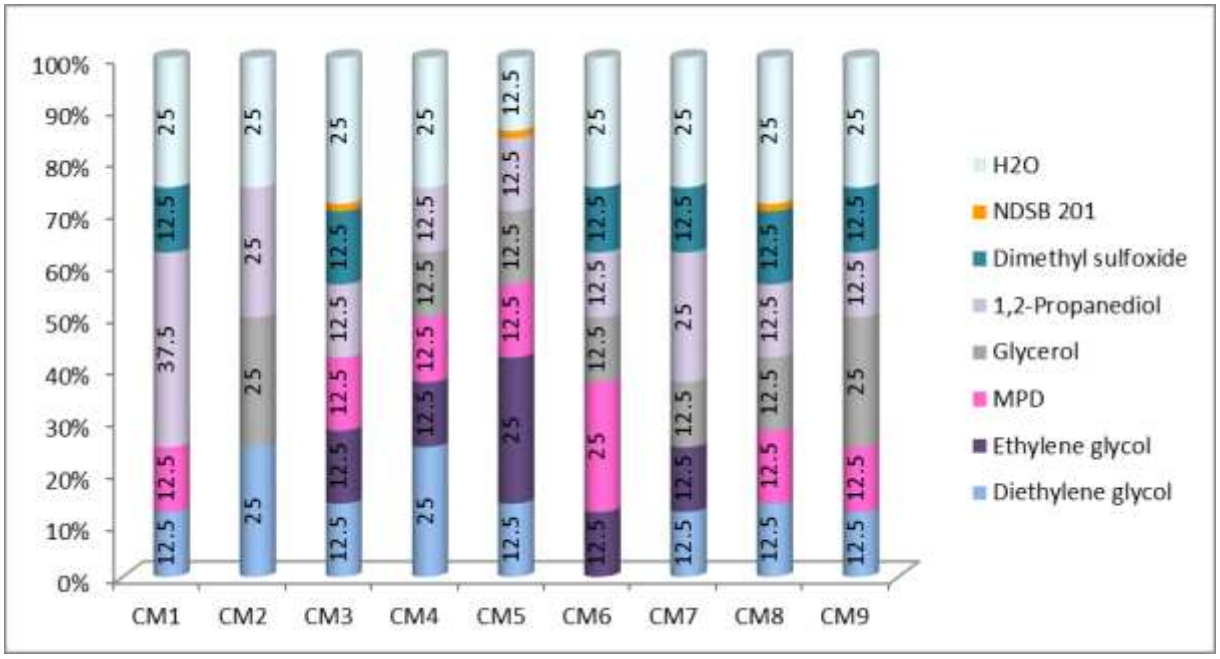

Figure1. The composition of the nine starting CryoMix (CM) formulations.

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Background Information

CryoProtX™ (Table 1) contains the following 6 groups:

- **CryoMixes™:** These mixes consist of multi-component formulations of cryoprotectants(Figure 3) which are also useful precipitants in protein crystallization. The CryoMixes™ induce small "reproducible" changes in dehydration since they contain less water than the solution from which the crystals have been grown.

- **Buffer Components:** Consist of broad range buffers (from the Really Useful Buffer Kit). The linearity of these buffer systems allows rapid preparation of buffered cryoprotectant solutions (Table 2).

- **Salt Components (Precipitant):** Consists of concentrated salts and a salt mixture. The dehydration effect provided by salts during cryoprotection may contribute to improvements in resolution. These salt components can be used in their pure form or can be mixed and tested unbuffered or with a buffer at various pHs.

- **PEG Components (Precipitant):** The presence of these cryo-precipitants counteracts the effect of the 'cryo-solubilizers' (glycerol, ethylene andpropylene glycols). Short polyethylene glycols such as PEG 400 and PEG 550 MME have been included along with the longer chain PEGs. These PEG solutions can be used in their pure form or as a mixture and can be tested unbuffered or with a buffer at various pHs.

- **Core Components (Figure 2):** These components have been chosen because of their suitability towards keeping crystals stable for long periods of time. These are provided for use during fine-tuning a cryoprotectant during customization steps.

- **Sugar Components (Additive):** The sugar mixes have been supplied to allow greater flexibility of design of your cryoprotectant solution. Addition of these may improve longevity at synchrotron radiation sources.

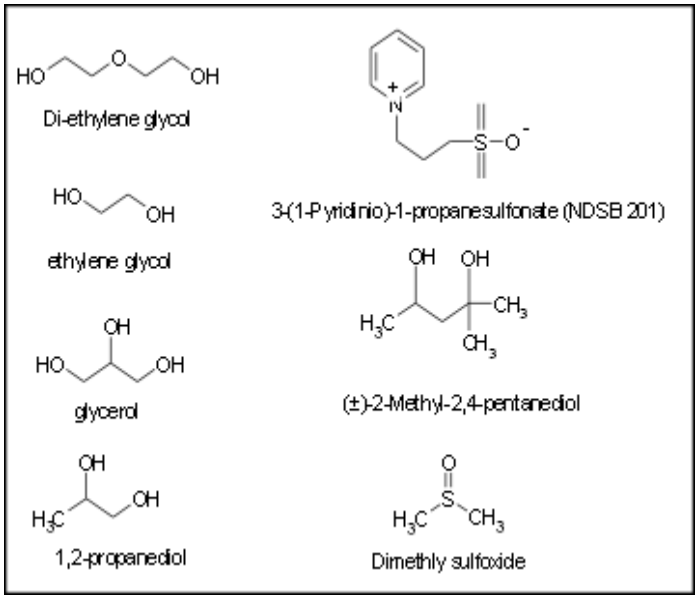

Figure 2. Core Components used in CryoProtX™

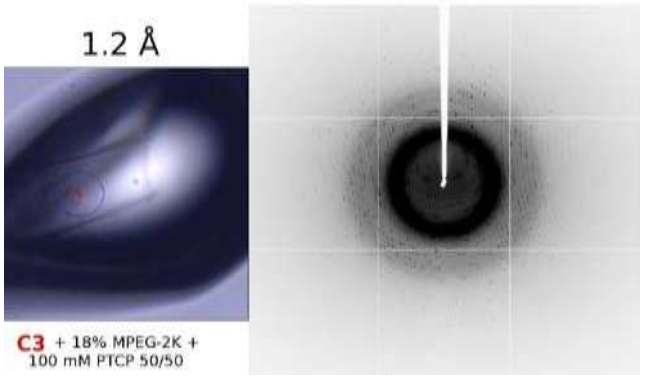

Figure 3: An example of quality cryoprotection achieved using solution C9 from CryoProtX™, buffer PCTP at pH 7.0 from The Really Useful Buffer Kit and a final precipitant concentration of 18% MPEG-2K mixed..

Notes/hints and tips:

Cryoprotect your crystal using a suitable method or follow this method:
In order to prevent over-dehydrating your crystal we recommend you **rapidly** transfer your crystal from the crystallization tray to the cryoprotectant.

Loop out the crystal from the crystallization tray and deposit the crystal in the cryoprotectant solution in either a cryo-tray or microbridge prepared earlier.
For short duration soaking (<15 minutes) we recommend that you leave the crystal in the cryoprotectant exposed to ambient air.
For longer soaks - that allow diffusion of ligands into the crystal lattice, we recommend temporarily covering your experiment.

Your crystal is now ready to be flash-cooled. Both transfers from crystallization tray to cryoprotectant solution and to liquid nitrogen should be carried out rapidly.
The first transfer - from crystallization tray to cryoprotectant is more critical than the second.

Capillary transfer method:

Attach a glass capillary to a small syringe.
Pick up crystal from drop in which they were grown.
Transfer into the cryoprotectant solution together with a small amount of mother liquor.
The suggested ratio of mother liquor to cryoprotectant solution is 1:5.
If larger cryoprotectant volumes are used this ratio can be increased to 1:50.
Transfers using this technique can reduce crystal 'shock' but may take longer to perform.
This method has the advantage that it can preserve important additives (even at low concentrations) that would be difficult to add to cryosolutions using other techniques.

Formulation Notes:

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

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

Enquiries regarding CryoProtX™ 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
Manufacturer's safety data sheets are available to download from our website.

References

Vera, Laura, and Enrico A. Stura. "Strategies for protein cryocrystallography." *Crystal Growth & Design* 14.2 (2013): 427-435.

Holyoak, T., Fenn,T. D., Wilson, M. A, Moulin, A. G., Ringe D., Petsko G. A. Malonate: *A versatile cryoprotectant and stabilizing solution for salt-grown macromolecular crystals. Acta Cryst D, 2003, 59, 2356–2358.*

Newman, J. *Novel buffer systems for macromolecular crystallization. Acta Cryst D, 2004, 60, 610-612.*

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Table 1 CryoProtX™ MD1-61

Tube #	Conc.	Units		Conc.	Units		Conc.	Units		Conc.	Units		Conc.	Units	
Red caps															
1	12.5	% v/v	Diethylene glycol	12.5	% v/v	MPD	37.5	% v/v	1,2-Propanediol	12.5	% v/v	Dimethyl sulfoxide			
2	25	% v/v	Diethylene glycol	25	% v/v	Glycerol	25	% v/v	1,2-Propanediol						
3	12.5	% v/v	Diethylene glycol	12.5	% v/v	Ethylene glycol	12.5	% v/v	MPD	12.5	% v/v	1,2-Propanediol	12.5	%v/v	Dimethyl sulfoxide
4	25.0	% v/v	Diethylene glycol	12.5	% v/v	Ethylene glycol	12.5	% v/v	MPD	12.5	% v/v	1,2-Propanediol	12.5	%v/v	Glycerol
5	12.5	% v/v	Diethylene glycol	25	% v/v	Ethylene glycol	12.5	% v/v	MPD	12.5	% v/v	1,2-Propanediol	12.5	%v/v	Glycerol
6	12.5	% v/v	Ethylene glycol	25	% v/v	MPD	12.5	% v/v	1,2-Propanediol	12.5	% v/v	Dimethyl sulfoxide	12.5	%v/v	Glycerol
7	12.5	% v/v	Diethylene glycol	12.5	% v/v	Ethylene glycol	25	% v/v	1,2-Propanediol	12.5	% v/v	Dimethyl sulfoxide	12.5	%v/v	Glycerol
8	12.5	% v/v	Diethylene glycol	12.5	% v/v	MPD	12.5	% v/v	1,2-Propanediol	12.5	% v/v	Dimethyl sulfoxide	12.5	%v/v	Glycerol
9	12.5	% v/v	Diethylene glycol	12.5	% v/v	MPD	12.5	% v/v	1,2-Propanediol	12.5	% v/v	Dimethyl sulfoxide	25	%v/v	Glycerol

Tube #	Conc.	Units		Conc.	Units		Conc.	Units	
Yellow caps									
1	1.0	M	MIB pH 4.0						
2	1.0	M	MIB pH 10.0						
3	1.0	M	PCTP pH 4.0						
4	1.0	M	PCTP pH 9.5						
5	1.0	M	MMT pH 4.0						
6	1.0	M	MMT pH 9.0						
7	1.0	M	CHC pH 4.0						
8	1.0	M	CHC pH 10.0						
9	1.0	M	AAB pH 4.0						
10	1.0	M	AAB pH 9.0						
Pink caps									
1	2.5	M	Lithium sulfate						
2	2.5	M	Lithium formate monohydrate						
3	0.3	M	Sodium malonate dibasic monohydrate	0.3	M	Sodium sulfate	0.3	M	Sodium formate
4	2.5	M	Sodium malonate dibasic monohydrate						
5	1.0	M	Sodium sulfate						
6	2.5	M	Sodium formate						

Green caps			
1	100	% v/v	PEG 400
2	50	% v/v	PEG 500 MME
3	50	% w/v	PEG 1000
4	50	% w/v	PEG 3350
5	50	% w/v	PEG 5000 MME
6	50	% w/v	PEG 8000
7	50	% w/v	PEG 10000
Blue caps			
1	50	% v/v	Diethylene glycol
2	100	% v/v	Ethylene glycol
3	100	% v/v	Glycerol
4	100	% v/v	MPD
5	100	% v/v	1,2-Propanediol
6	100	% v/v	Dimethyl sulfoxide
7	100	mM	NDSB 201

Tube #	Conc.	Units		Conc.	Units		Conc.	Units	
Clear/white caps									
1	0.3	M	D-Trehalose	0.3	M	Sucrose	0.3	M	D-Maltose
2	0.3	M	Xylitol	0.3	M	D-Glucose			
3	30	% w/v	D-Trehalose						
4	30	% w/v	Sucrose						
5	30	% w/v	D-Maltose						
6	30	% w/v	Xylitol						
7	30	% w/v	D-Glucose						

Abbreviations:
MPD: (2-Methyl-2,4-pentanediol); **PEG**: (Polyethylene Glycol); **DMSO** (dimethyl sulfoxide); **NDSB-201**; (non detergent sulfobetaine, 3-(1-Pyridino)-1-propane sulfonate); **PEG MME**; (Polyethylene glycol monomethyl ether); **MIB buffer**: (Sodium malonate dibasic monohydrate, Imidazole, Boric acid); **PCTP Buffer**: (Sodium propionate, sodium cacodylate trihydrate, Bis-Tris propane); **MMT Buffer**: (DL-Malic acid, MES monohydrate, Tris); **CHC Buffer**: (Citric acid, HEPES, CHES); **AAB Buffer**: Sodium acetate trihydrate, ADA, BICINE)

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

Table 2. Preparation of Buffer Components

For MIB and CHC buffers		
Desired pH (approx.)	Volume of pH 4	Volume of pH 10
4	1000	0
5	835	165
6	670	330
7	500	500
8	330	670
9	165	835
10	0	1000
For PCTP buffer		
Desired pH (approx.)	Volume of pH 4	Volume of pH 9.5
4	1000	0
5	835	165
6	670	330
7	500	500
8	330	670
9	165	835
9.5	0	1000
For MMT and AAB Buffers		
Desired pH (approx.)	Volume of pH 4	Volume of pH 9
4	1000	0
5	800	200
6	600	400
7	400	600
8	200	800
9	0	1000

The above volumes are all approximate and given in μL for a total volume of 1000 μL. Adjust total volume as necessary.

Re-Ordering details:

CryoProtX™ (46 x 1.5 mL)	MD1-61
CryoSol™	MD1-90
Cryo Combination (CryoProtX™ + CryoSol™)	MD1-94

CryoProtX™ Mixes (1.5 mL)

CryoMix™ 1	MDSR-61-CM1
CryoMix™ 2	MDSR-61-CM2
CryoMix™ 3	MDSR-61-CM3
CryoMix™ 4	MDSR-61-CM4
CryoMix™ 5	MDSR-61-CM5
CryoMix™ 6	MDSR-61-CM6
CryoMix™ 7	MDSR-61-CM7
CryoMix™ 8	MDSR-61-CM8
CryoMix™ 9	MDSR-61-CM9
Salt Mix 3	MDSR-61-Salt3
Sugar Mix 1	MDSR-61-SM1
Sugar Mix 2	MDSR-61-SM2
The Really Useful Buffer Kit (10 mL)	MD2-101

All other reagents and individual buffers can be ordered as standard stock reagents (100 mL or 250 mL). See our website for details.

CryoProtX™ (MD1-61)

Quick-Start Guide

- These CryoMixes are intended for single-step cryosoaking.
- Test all nine of the CryoMixes supplied using small, low value crystals of your protein of interest (Option 1).
- Further Options are shown. These are just suggestions of what to try with your protein crystal.

Suggested Method of Use

(coloured dots correspond to colour of vials suggested for use in that option).

1. Test all nine CryoMix solutions- (see flow-chart p2).

Mixing your own CryoSolutions (Options 2 -5):

Use 40 µL CryoMix + 10 µL Buffer + 50 µL water, salt/PEG/sugar*.

2. CryoMix + Buffer

3. CryoMix + Buffer + Salt or PEG

4. CryoMix + Buffer + Salt + PEG

5. CryoMix + Buffer + Salt + PEG + Sugar

*The PEG/salt/sugar volume is typically mixed using a variable volume of stock with a complementary volume of water to add up to 50 µL.

Buffers are made up to corresponding pH's as shown in the Buffer Table (p3).

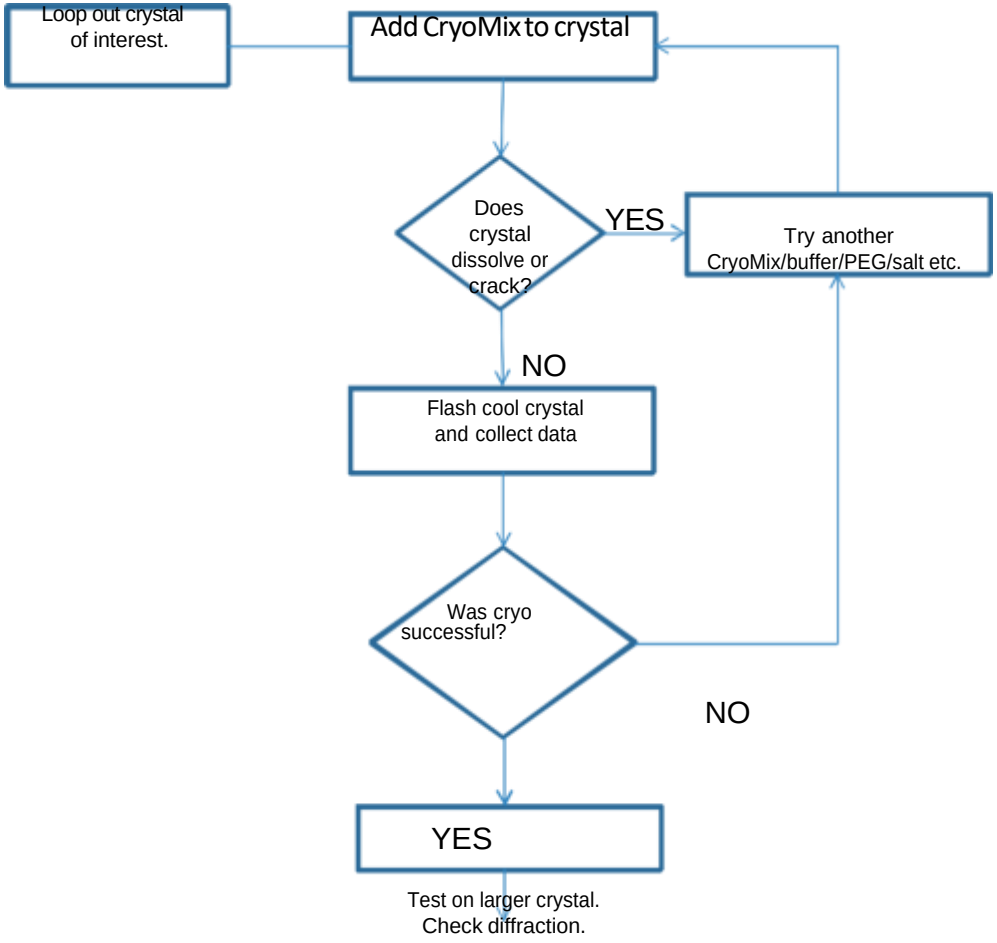
Advanced Method

Mix your own CryoMix from the CryoMix components + any of the Buffers, Salts, PEGs or sugars to make your own customized cryoprotectant.

+ Component/s of choice

CryoProtX™ (MD1-61)

Quick-Start Guide



See Customization Tips forfurther experiments (p4)

CryoProtX™ (MD1-61)

Quick-Start Guide

Preparation of Buffer Components

For MIB and CHC buffers		
Desired pH (approx.)	Volume of pH 4	Volume of pH 10
4	1000	0
5	835	165
6	670	330
7	500	500
8	330	670
9	165	835
10	0	1000
For PCTP buffer		
Desired pH (approx.)	Volume of pH 4	Volume of pH 9.5
4	1000	0
5	835	165
6	670	330
7	500	500
8	330	670
9	165	835
9.5	0	1000
For MMT and AAB Buffers		
Desired pH (approx.)	Volume of pH 4	Volume of pH 9
4	1000	0
5	800	200
6	600	400
7	400	600
8	200	800
9	0	1000

The above volumes are all approximate and given in μL for a total volume of 1000 μL. Adjust total volume as necessary.

CryoProtX™ (MD1-61)

Quick-Start Guide

Customization Guide & Tips:

- These CryoMixes are intended for single-step cryosoaking.
- Test all nine of the CryoMixes supplied using small, low value crystals of your protein of interest.
- If no conditions work with the CryoMixes, repeat using different buffer/PEG/salt etc.
- If larger crystals have problems, then the transfer method should be changed from loop transfer to capillary transfer method (See Page 3 of main datasheet).
- Diffraction tests - *when diffraction, mosaicity, longevity in the synchrotron beam is optimized.* The two best diffracting solutions from the initial CryoMix tests can be combined together (50:50) and mixed 40 µL new CryoMix/ 50 µL PEG /10 µL buffer.
- The addition of extra additives, such as salts or sugar mixes, could improve the resolution of your crystal even further. Additives like sugars can be introduced once a suitable working cryoprotectant solution has been found. Sugar components can be used as additives to replace some of the water/precipitant.
- Further optimization can be attempted by creating new mixes using alternative CryoMix components , salts, PEGs and sugars.
- For high ionic strength crystallizations, try 80% saturated lithium sulfate in your cryoprotectant mix.
- Cryosolutions do not need to be related to the precipitants the crystals were grown is as long as they dehydrate the crystals.
- For DMSO containing CryoMixes, drug/small molecule ligand screening with low affinity inhibitors should be facilitated, as it is possible to add extra inhibitor without the inhibitor precipitating out in the cryo solution.

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CryoSol™ MD1-90

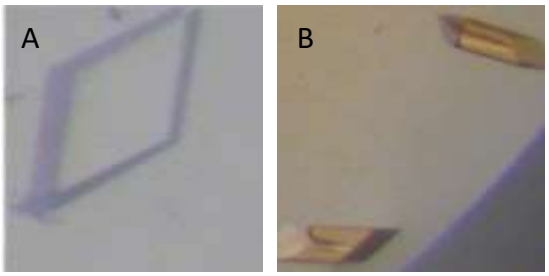
A set of multicomponent solutions intended for ligand solubilisation/soaking of hydrophobic ligands into crystals and subsequent crystal cryoprotection.

Developed in the Laboratory of Dr. Enrico Stura at CEA Saclay, France.

MD1-90 is presented as 33 x 1.5 mL microtubes (18 x multi-component cryoprotectant solutions (CM1-18), 6 x solubilisation solutions (SM1-6) and 9 x stock solutions).

Features of CryoSol™:

- Solubilization of hydrophobic ligands.
- Easily prepare ligand-loaded cryosolutions for soaking experiments.
- Co-crystallization screening of protein-ligand complexes.
- Protein friendly mixes of Dimethyl sulfoxide (DMSO/dioxane/ethylene glycol should improve ligand solubilization.
- Contains 2,3-butanediol- compatible with enzymatic activity.



Crystal photos from ICCBM 15 poster presentation.
Photomicrographs of crystals of wild-type human transthyretin (TTR) grown in the presence of various ligands. (A) Crystals of the TTR-16α-bromo-estradiol complex. The crystals are grown with a lower concentration of PEG. PEG can participate in the solubilization of the steroid, but the small change in concentration has almost no influence on ligand crystallization. (B) Crystals of TTR grown in the presence of curcumin at pH7.4

Introduction

CryoSol™ is a set of multicomponent solutions for ligand solubilisation and soaking using Solubilization/cryoprotection Mixtures 1-6 (SM1-SM6) and crystal cryoprotection with CryoMixes™ 1 -18 (CM1-CM18), are shown in Figure 1.
The compositions of SM1-SM6 and CM1-CM18 include dioxane and butanediol as well as DMSO. Whilst high DMSO concentrations can be detrimental to proteins, high dioxane concentrations on the other hand, are well tolerated.

Dioxane is also effective for ligand solubilization alone and in conjunction with other organic compounds with cryoprotectant properties.
This kit is intended for ligand solubilization to prepare protein-ligand complexes for co-crystallization screening. It is also intended for the preparation of ligand-loaded cryosolutions for soaking into crystals.

Dioxane and glycols have opposite effects on protein solubility and can be exploited to improve crystals. Dioxane increases the precipitating power of the reservoir solution in vapour diffusion experiments. Dioxane-PEG mixtures allow the crystallization of proteins that are not precipitated by PEG alone. Dioxane is also a common precipitant/additive in protein crystallization.

Multicomponent cryoprotectant solutions (CM1-CM18)

CryoMixes™ CM1- CM18 are an extended set (in addition to those provided in CryoProtX™, MD1-61) of multicomponent solutions for crystal cryoprotection and single-step cryosoaking and includes additional components, dioxane and butanediol not found in CryoProtX™, making these conditions more suitable for working with ligands not usually soluble in standard cryosolutions.

Ligand Solubilization (SM1-6)

SM1-6 mixtures (Figure 1) are intended for the solubilization of hydrophobic ligands for macromolecular co-crystallization and crystal soaking experiments. The mixed solutions with DMSO/dioxane/ethylene glycol at different ratios allow a more comprehensive ligand solubilisation because of co-solvency. The individual compounds cover a relatively wide range of selectivity values so their combination is appropriate to achieve high ligand concentrations for a large variety of organic compounds in an aqueous medium. The mixed compounds avoid the use of excessive concentrations of organic solvents incompatible with enzyme activity.

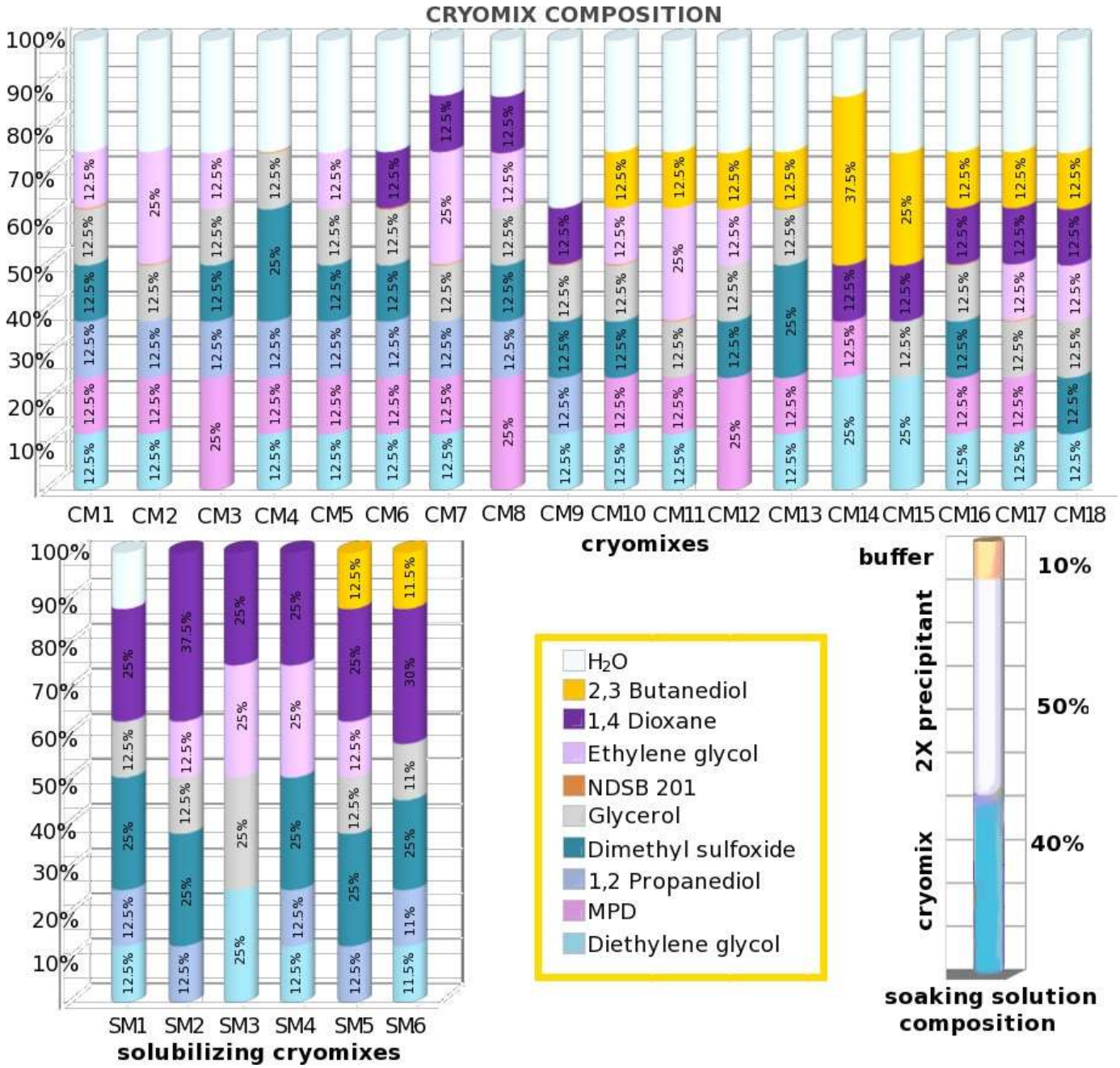


Figure 1: Composition of the six ligand solubilizing mixed cryosolutions **SM1- SM6,** shown graphically as cylinders. Composition of the 18 CryoMixes™ (**CM1-CM18**) and composition of the cryo ligand soaking solution (bottom right).

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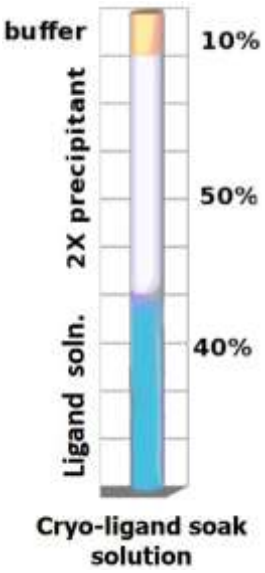
CryoSol Quick Start Guide for Ligand Soaking with Cryoprotection:

N.B. See Figure 3 for a flowchart of how best to use CryoSol™.

1. Dissolve ligand in DMSO, Dioxane or SM1-6 up to a concentration of 100mM. This is the **ligand solution**.
2. Then, to make a **cryoprotected ligand solution**, do the following:
   If your ligand was dissolved in:
   a) **DMSO** - dilute with 75% SM3 (or any CM not containing DMSO, e.g. CM2, 7, 11, 14, 15 or 17) e.g. 25% ligand solution + 75% SM3.
   or, b) **Dioxane** - dilute with 50% of any SM solution (or any CM), e.g. 50% ligand solution: 50% SM 1-6 or CM,
   or, c) **SM1-6** - go directly to Step 3 without any further dilution.
   Check that ligand remains dissolved – any problems, return to step 1 and try another condition.
3. Make-up the final **cryo-ligand soaking solution** (Figure 2) with cryoprotected ligand solution by adding precipitant and buffer (these are from the crystallization hit).
   For example to make up 10 µL cryo ligand soaking solution: Take
   - 4µl cryoprotected ligand solution
   - 5µl 2x precipitant mix (e.g. if you had 0.1M ppt in crystallization hit, then you would use 0.2M).
   - 1µl 10x buffer (if you had 0.1M in your crystallization hit, then you would use 1M).
4. Soak Crystal for 1 – 20 minutes in the cryo-ligand soaking solution.
5. Collect data.
6. To improve resolution and/or reduce mosaicity, you can return to step 2 (or step 1 if necessary) to try out different cryomix combinations.

Figure 2: To formulate the **cryo-ligand soak solution**, the cryo-protected ligand solution represents 40% of the volume, 10% to the buffer (used in your crystallization experiment) and 50% to the precipitant-water mixture that is 2X the concentration of the crystallization precipitant (used in your crystallization experiment).



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See Figure 3 for a flowchart of ligand solubilisation and cryoprotectant procedure.

Tips & Hints

Ligand solubilization (SM1-6)

Target final ligand concentrations for soaking experiments should be in the range of 1 – 10 mM for ligands with high micromolar affinity.

Solubilizing the ligand in DMSO:

Aim to have 10% DMSO or less in the final soaking solution or protein solution for crystallization trials. Use solution SM3 when making up the soaking solution.

Solubilizing the ligand in Dioxane:

Aim for 20% (30% max.) dioxane in the final solution. All SM1-6 can be used for the dilution of the dioxane-ligand solution. The DMSO containing SM mixes should be favoured as the dioxane-DMSO combination will provide better ligand solubilisation.

Use in soaking experiments:

The additional components of the SM solutions should maintain the ligand in solution whilst reducing potentially damaging levels of DMSO.
If during soaking experiments the ligand precipitates out or forms crystals, then use larger volumes and lower ligand concentrations.

Use in ligand exchange experiments:

Make the ligand solutions as for soaking experiments. Allow from 20 minutes to overnight for ligand exchange. Successive movements ofthe crystals from one "new" ligand solution to another will help eliminate the "old" ligand from the crystals. Do this every 20 mins to accelerate the exchange. This is important when the "old" ligand has comparable affinity to the "new" one. Successive exchanges with high concentrationsof the "new" ligand will be more effective than trying to wash out the "old" ligand first.

Using in crystallization experiments

Make a ligand-protein solution with ligand solubilised in a DMSO/dioxane mixture or any SM1-6. Add up to 10% DMSO solubilized ligand, 20% dioxane solubilized ligand. **When screening, ensure that the compounds used to solubilize your ligand are added to the precipitants in the reservoir. These compounds are hygroscopic and will prevent proper equilibration without this precaution. Screen for crystals in the usual manner.**

Cryo-protecting ligand-complex co-crystals (CM1-18):

Solutions CM1-18 are designed specificallyto help aid cryoprotection of protein-ligand complex co-crystals. They provide cryoprotection to an equivalent of 40% glycerol without causing damage to your crystal, but also help maintain enzymatic activity. Make up as shown in Figure 2, using chosen CM solution in place of ligand solution. These solutions could also be used in place of SM1-6 during a ligand soak if the ligand is solubilized in DMSO or dioxane e.g. for a ligand exchange.

References

Ciccone L, Tepshi L, Nencetti S, Stura EA.N Biotechnol. (2015) *"Transthyretin complexes with curcumin and bromo-estradiol: Evaluation of solubilizing multicomponent mixtures"* 32(1):54-64.

L Vera, EA Stura - Crystal Growth & Design, (2014) *"Strategies for Protein Cryocrystallography"*,14 (2), 427-435.

Ciccone L., Vera L., Tepshi L., Rosalia L., Rossello A. & Stura E.A. "Multicomponent mixtures for cryoprotection and ligand solubilization." *Biotechnology Reports* 7 (2015): 120-127. Open Access.

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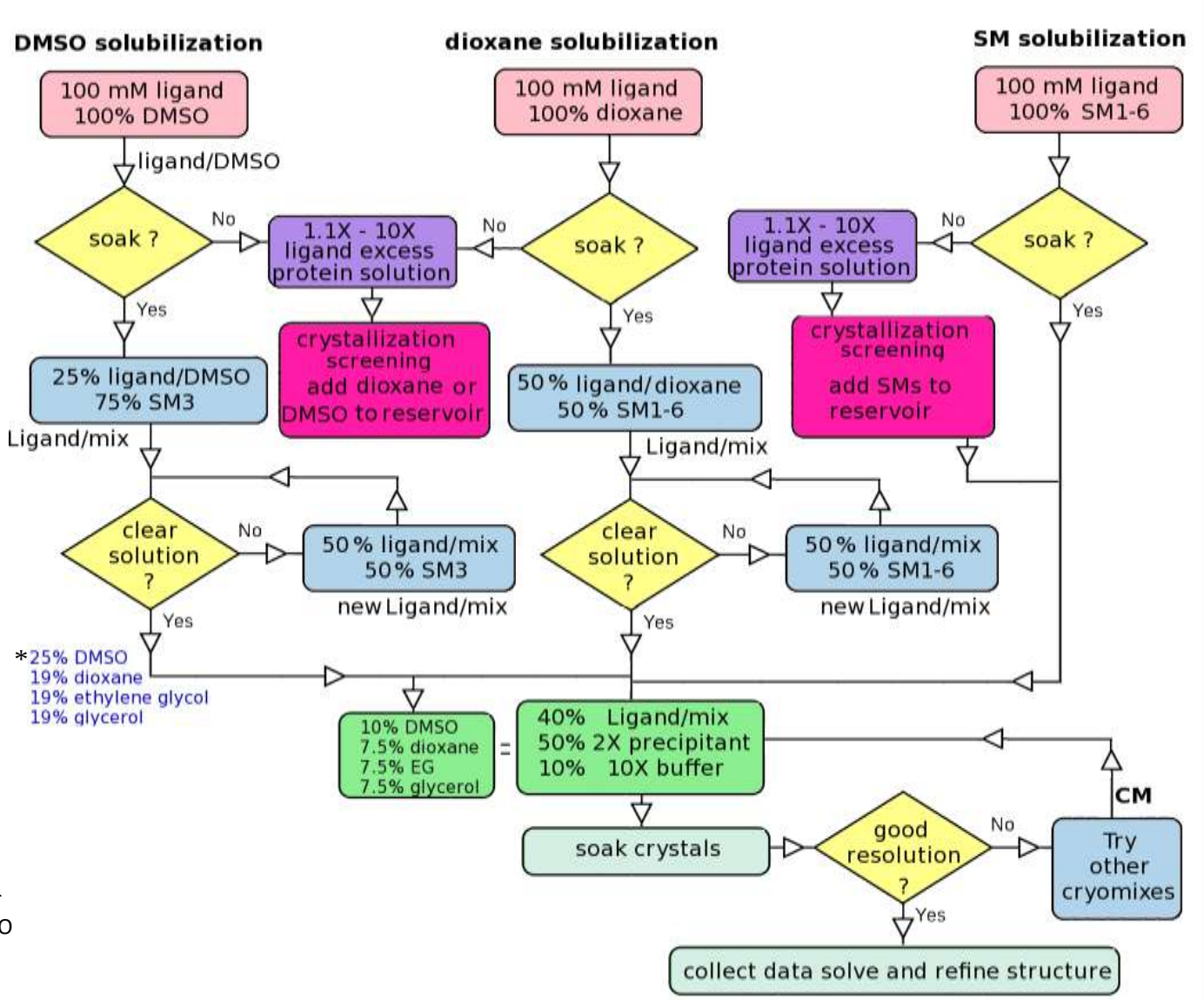
Figure 3 - Flow chart showing an overview of the ligand solubilisation and cryoprotectant procedure



*Contents of solution after diluting 25% ligand in DMSO + 75% SM3

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CryoSol™

MD1-90

Tube ID	Conc.	Reagent 1	Conc.	Reagent 2	Conc.	Reagent 3	Conc.	Reagent 4	Conc.	Reagent 5	Conc.	Reagent 6	Conc.	Reagent 7
Red caps														
CM1	12.5 % v/v	Diethylene glycol	12.5 % v/v	MPD	12.5 % v/v	1,2-Propanediol	12.5 % v/v	Dimethyl sulfoxide	12.5 % v/v	Glycerol	12.5 mM	NDSB 201	12.5 % v/v	Ethylene glycol
CM2	12.5 % v/v	Diethylene glycol	12.5 % v/v	MPD	12.5 % v/v	1,2-Propanediol	12.5 % v/v	Glycerol	12.5 mM	NDSB 201	25 % v/v	Ethylene glycol		
CM3	25 % v/v	MPD	12.5 % v/v	1,2-Propanediol	12.5 % v/v	Dimethyl sulfoxide	12.5 % v/v	Glycerol	12.5 % v/v	Ethylene glycol				
CM4	12.5 % v/v	Diethylene glycol	12.5 % v/v	MPD	12.5 % v/v	1,2-Propanediol	25 % v/v	Dimethyl sulfoxide	12.5 % v/v	Glycerol	12.5 mM	NDSB 201		
CM5	12.5 % v/v	Diethylene glycol	12.5 % v/v	MPD	12.5 % v/v	1,2-Propanediol	12.5 % v/v	Dimethyl sulfoxide	12.5 % v/v	Glycerol	12.5 % v/v	Ethylene glycol		
CM6	12.5 % v/v	Diethylene glycol	12.5 % v/v	MPD	12.5 % v/v	1,2-Propanediol	12.5 % v/v	Dimethyl sulfoxide	12.5 % v/v	Glycerol	12.5 mM	NDSB 201	12.5 % v/v	1,4-Dioxane
CM7	12.5 % v/v	Diethylene glycol	12.5 % v/v	MPD	12.5 % v/v	1,2-Propanediol	12.5 % v/v	Glycerol	12.5 mM	NDSB 201	25 % v/v	Ethylene glycol	12.5 % v/v	1,4-Dioxane
CM8	25 % v/v	MPD	12.5 % v/v	1,2-Propanediol	12.5 % v/v	Dimethyl sulfoxide	12.5 % v/v	Glycerol	12.5 % v/v	Ethylene glycol	12.5 % v/v	1,4-Dioxane		
CM9	12.5 % v/v	Diethylene glycol	12.5 % v/v	1,2-Propanediol	12.5 % v/v	Dimethyl sulfoxide	12.5 % v/v	Glycerol	12.5 mM	NDSB 201	12.5 % v/v	1,4-Dioxane		
CM10	12.5 % v/v	Diethylene glycol	12.5 % v/v	Ethylene glycol	12.5 % v/v	MPD	12.5 % v/v	Glycerol	12.5 % v/v	2,3-Butanediol	12.5 % v/v	Dimethyl sulfoxide	12.5 mM	NDSB 201
CM11	12.5 % v/v	Diethylene glycol	25 % v/v	Ethylene glycol	12.5 % v/v	MPD	12.5 % v/v	Glycerol	12.5 % v/v	2,3-Butanediol	12.5 mM	NDSB 201		
CM12	12.5 % v/v	Ethylene glycol	25 % v/v	MPD	12.5 % v/v	Glycerol	12.5 % v/v	2,3-Butanediol	12.5 % v/v	Dimethyl sulfoxide				
CM13	12.5 % v/v	Diethylene glycol	12.5 % v/v	MPD	12.5 % v/v	Glycerol	12.5 % v/v	2,3-Butanediol	25 % v/v	Dimethyl sulfoxide	12.5 mM	NDSB 201		
CM14	25 % v/v	Diethylene glycol	12.5 % v/v	MPD	37.5 % v/v	2,3-Butanediol	12.5 % v/v	1,4-Dioxane						
CM15	25 % v/v	Diethylene glycol	12.5 % v/v	Glycerol	25 % v/v	2,3-Butanediol	12.5 % v/v	1,4-Dioxane						
CM16	12.5 % v/v	Diethylene glycol	12.5 % v/v	MPD	12.5 % v/v	Glycerol	12.5 % v/v	2,3-Butanediol	12.5 % v/v	Dimethyl sulfoxide	12.5 % v/v	1,4-Dioxane	12.5 mM	NDSB 201
CM17	12.5 % v/v	Diethylene glycol	12.5 % v/v	Ethylene glycol	12.5 % v/v	MPD	12.5 % v/v	Glycerol	12.5 % v/v	2,3-Butanediol	12.5 % v/v	1,4-Dioxane	12.5 mM	NDSB 201
CM18	12.5 % v/v	Diethylene glycol	12.5 % v/v	Ethylene glycol	12.5 % v/v	Glycerol	12.5 % v/v	2,3-Butanediol	12.5 % v/v	Dimethyl sulfoxide	12.5 % v/v	1,4-Dioxane		
Purple caps														
SM1	12.5 % v/v	Diethylene glycol	12.5 % v/v	Glycerol	12.5 % v/v	1,2-Propanediol	25 % v/v	Dimethyl sulfoxide	25 % v/v	1,4-Dioxane				
SM2	12.5 % v/v	Ethylene glycol	12.5 % v/v	Glycerol	12.5 % v/v	1,2-Propanediol	25 % v/v	Dimethyl sulfoxide	37.5 % v/v	1,4-Dioxane				
SM3	25 % v/v	Diethylene glycol	25 % v/v	Ethylene glycol	25 % v/v	Glycerol	25 % v/v	1,4-Dioxane						
SM4	12.5 % v/v	Diethylene glycol	25 % v/v	Ethylene glycol	12.5 % v/v	1,2-Propanediol	25 % v/v	Dimethyl sulfoxide	25 % v/v	1,4-Dioxane				
SM5	12.5 % v/v	Ethylene glycol	12.5 % v/v	Glycerol	12.5 % v/v	1,2-Propanediol	12.5 % v/v	2,3-Butanediol	25 % v/v	Dimethyl sulfoxide	25 % v/v	1,4-Dioxane		
SM6	12.5 % v/v	Diethylene glycol	12.5 % v/v	Glycerol	12.5 % v/v	1,2-Propanediol	12.5 % v/v	2,3-Butanediol	20 % v/v	Dimethyl sulfoxide	30 % v/v	1,4-Dioxane		
Blue caps														
Stock1	50 % v/v	Diethylene glycol												
Stock2	100 %	Ethylene glycol												
Stock3	100 %	Glycerol												
Stock4	100 %	MPD												
Stock5	100 %	1,2-Propanediol												
Stock6	100 %	Dimethyl sulfoxide												
Stock7	100 mM	NDSB 201												
Stock8	100 %	1,4-Dioxane												
Stock9	100 %	2,3-Butanediol												

Molecular Dimensions

Abbreviations:
MPD: 2-methyl, 2,4-pentanediol, **NDSB 201:** 3-(1-Pyridinio)-1-propanesulfonate.

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

Re-Ordering details:

Catalogue Description		Catalogue Code
CryoSol™	(46 x 1.5 mL)	MD1-90
CryoProtX™	(33 x 1.5 mL)	MD1-61
The Cryo Combination (CryoSol™ + CryoProtX™)	(1 x CryoProtX™ + 1 x CryoSol™)	MD1-94

For CryoSol™ and CryoProtX™ stock reagents visit our Optimization page on our website.

Formulation Notes:

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

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

Enquiries regarding 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