Molecular Dimensions

CryoSol™ MD1-90

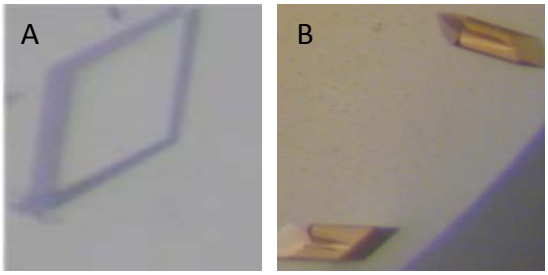
A set of multicomponent solutions intended for ligand solubilisation/soaking of hydrophobic ligands into crystals and subsequent crystal cryoprotection.

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.

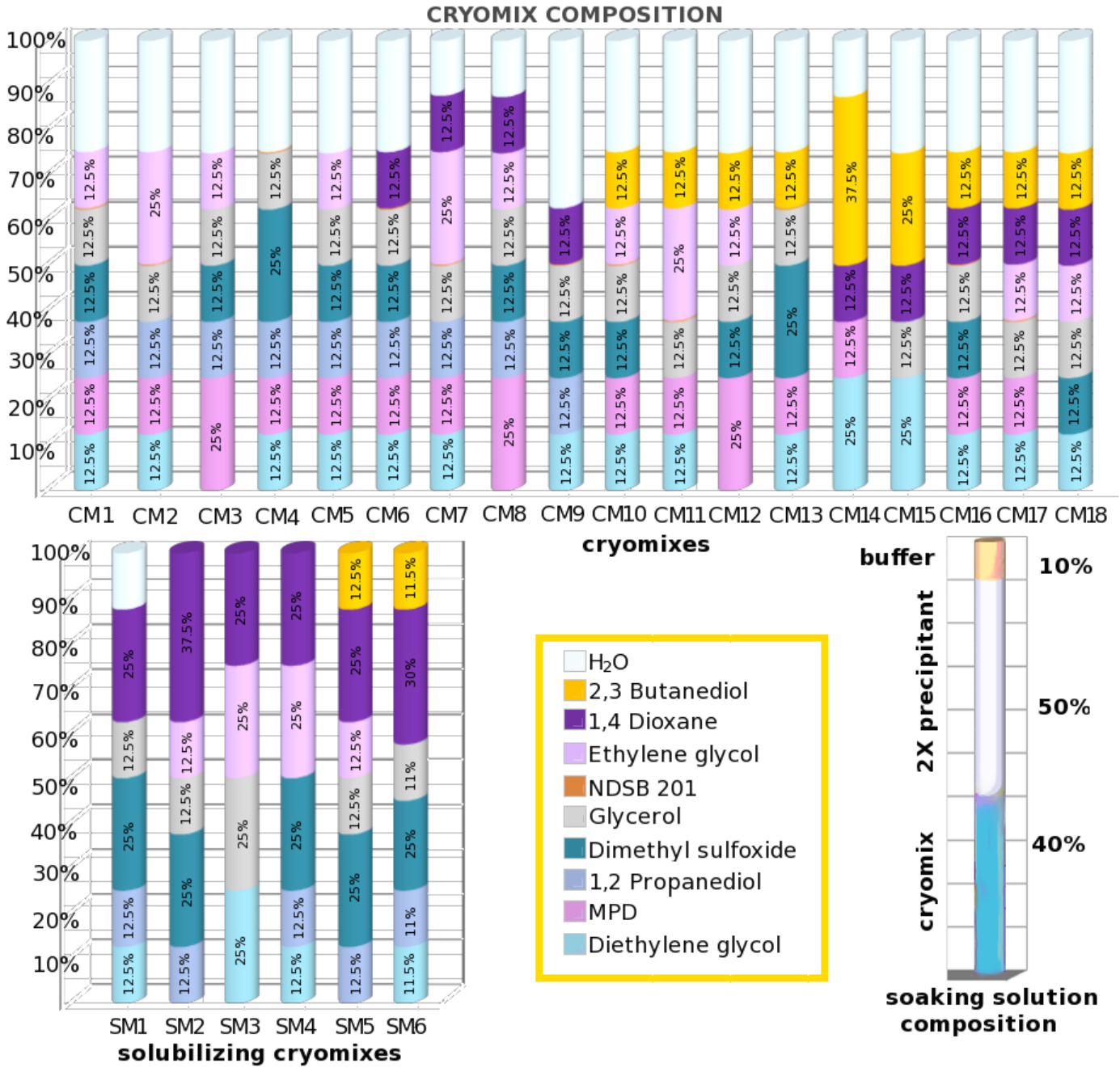


Figure 1: Composition of the six ligand solubilizing mixed cryosolutions **SM1- SM6,** shown graphically as cylinders. Composition of the 18 CryoMixes™ (**CM1-CM18**) and composition of the cryo ligand soaking solution (bottom right).

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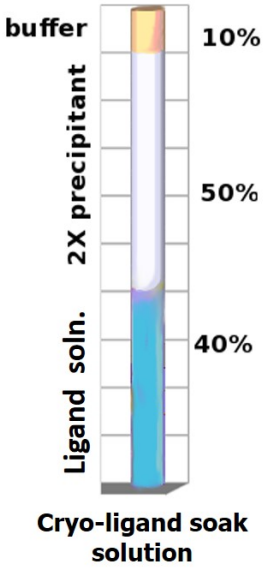
CryoSol Quick Start Guide for Ligand Soaking with Cryoprotection:

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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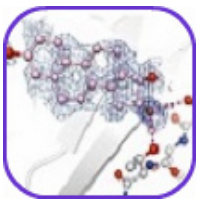

See Figure 3 for a flowchart of ligand solubilisation and cryoprotectant procedure.

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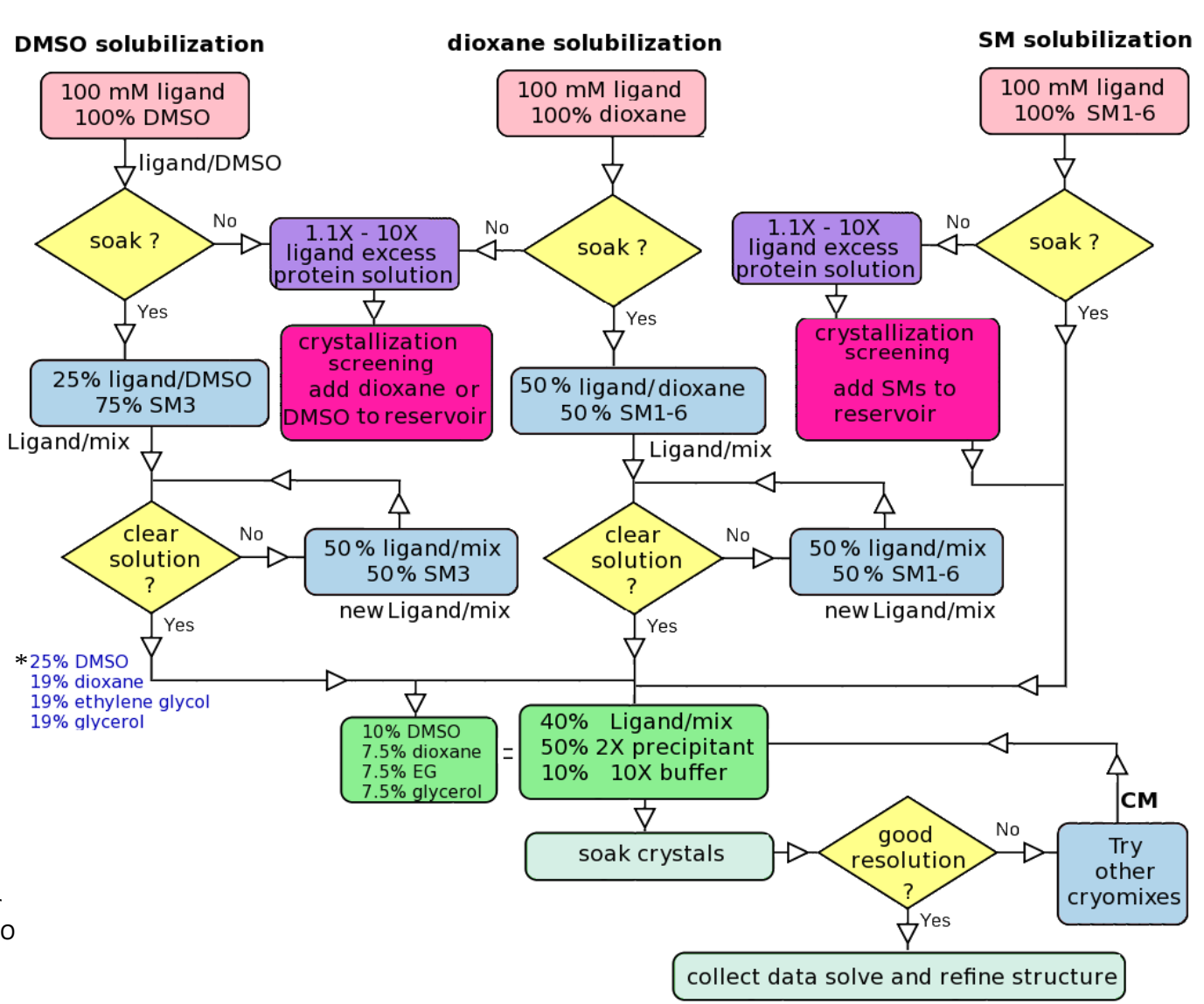
Figure 3 - Flow chart showing an overview of the ligand solubilisation and cryoprotectant procedure



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

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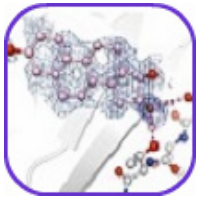

CryoSol™

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												

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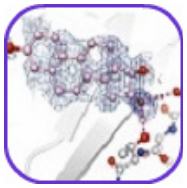

Abbreviations:
MPD: 2-methyl, 2,4-pentanediol, **NDSB 201:** 3-(1-Pyridinio)-1-propanesulfonate.

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