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

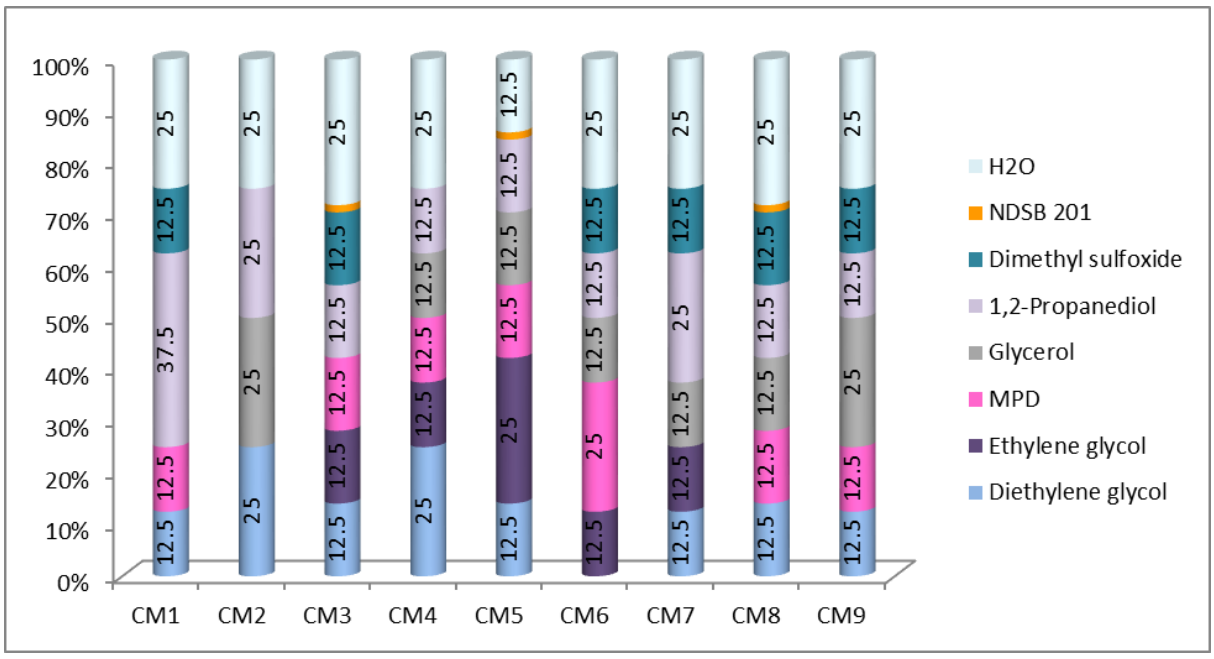

Figure1. The composition of the nine starting CryoMix (CM) formulations.

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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 and propylene 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.

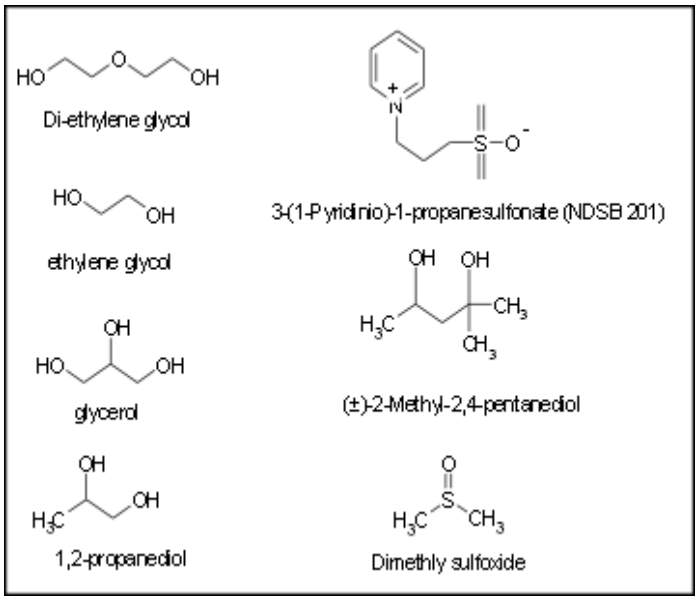

Figure 2. Core Components used in CryoProtX™

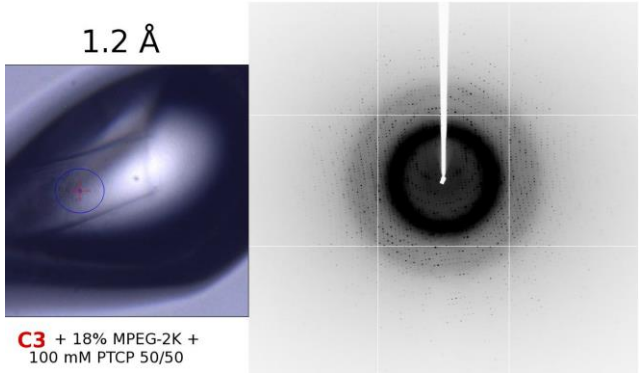

Figure 3: An example of quality cryoprotection achieved using solution C9 from CryoProtX™, buffer PCTP at pH 7.0 from The Really Useful Buffer Kit and a final precipitant concentration of 18% MPEG-2K mixed..

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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		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												

Tube #	Conc.	Units		Conc.	Units		Conc.	Units		Conc.	Units		Conc.	Units	
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												

Tube #	Conc.	Units		Conc.	Units		Conc.	Units		Conc.	Units		Conc.	Units	
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												

Tube #	Conc.	Units		Conc.	Units		Conc.	Units		Conc.	Units		Conc.	Units	
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		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.