Cryoprotectants

cryoprotectant	conc range%(w/v)	comments
glycerol	**13 - 35**	
ethylene glycol	**11 - 30**	
PEG 200	**25-35**	use 100% solution
PEG 400	**25 - 35**	use 100% solution
Xylitol	**22**	
(2R,3R)butane 2,3 diol	**8**	
Ethylenglykolmonoethylether	**10**	
Ethanol	**20% to reservoir, let equilibrate**	
Propanol	**20% to reservoir, let equilibrate**	
erythritol	**11**	
glucose	**25**	prepare 50%(w/v)stock,+N3, dissolves slowly!
sucrose	**25**	prepare 100%(w/v)stock,+N3, max solubil. 180g/100g H20,at 0 deg C
MPD	**15 - 40**	use 100% solution
KOac	**3.3 M of higher**	
NaCl	**5M**	
MgCl2	**2M or higher**	
NaFormiat	**6M**	

1. as first try to get an idea about the cryo-protectant concentration needed use your mother liquor and pipett the cryo protectant at certain percentage inside, freeze and take image (2 min exposure normally sufficient) (It may be necessary to dilute your reservoir 1:2, as the drop might have slightly more water)

2. then, prepare your reservoir solution with the chosen percentage of cryo-protectant

3. freeze and take an image

4. if conditions are o.k., try on the crystal;

5. take two pipettes, with one you withdraw the mother liquor, with the other you pipette the cryo-protectant containing buffer.

6. do this two times to remove all mother liquor

7. if crystal is stable in solution, freeze and put in beam

8. some crystals crack, if put in cryo-protectant, here try e.g. a stepwise increase in cryo-protectant

9. also, once the conditions for freezing are known, try if your protein crystallizes in cryobuffer!

Rhv. sulf NG domain cryo protocol:
grow Xtals by microseeding in

- 100 mM BISTRIS pH 6.0
- 200 mM CaOAc
- 20% PEG 8000
- 5 % MPD

transfer Xtals to 10% MPD in motherliquor (i.e. 50 ul + 950 ul)
shockfreeze in N2 - stream

Kai Tekaat
Last modified: Sun May 24 20:06:55 MDT 1998