



Protocol Section:	Cryoprotectant	Policy No:	P-CMMR4-CPA-00
Protocol Subject:	Sperm Freezing Cryoprotectant	Effective Date:	8 September 2005
		Date Reviewed:	22 September 2005
		Date Revised:	

Method taken from Harwell

- For all inbred strains except 129s and C57Bl/6, the CPA should be 18% raffinose and 3% skim milk.

CHEMICAL	SUPPLIER	50ml
Raffinose	Sigma R-0250	9.0g
Skimmed Milk Powder	Difco 232100	1.5g
Sterile Water		50ml

- Heat 50ml of water in a 50ml tube to 60°C in a water bath
- Add 9.0g of raffinose and dissolve by gentle inversion
- Add 1.5g skim milk and dissolve by gentle inversion
- Divide the mixture evenly into 2 tubes and centrifuge for 10mins at 14,000rpm
- Decant the supernatant into a fresh tube, discarding the pellet
- Filter sterilize the solution through a 0.45µm syringe filter
- Make 1.1ml aliquots in eppendorf tubes and store at -20°C for up to 3 months
- Ensure to date the tubes
- Osmolality should be between 470-490 mOsm

- For 129s and C57Bl/6 inbred strains, the CPA should be 0.2M raffinose and 0.1M glycerol. Protocol tested by Cengiz Yildiz
Osmolality should be between 430-450 mOsm

CHEMICAL	SUPPLIER	50ml
Raffinose	Sigma R-0250	5.945g
Skimmed Milk Powder	Difco 232100	1.5g
Glycerol	BP229-1	460µl
Sterile Water		50ml

Issued by Lab Manager: _____ **Date:** _____

Approved by Facility Management: _____ **Date:** _____