



Protocol Section:	Embryo Cryopreservation	Policy No:	P-CMMR1-ECD-01
Protocol Subject:	Modified Rapid Embryo Freezing Protocol D for ENU and Non-Enu Mice Embryos at Eight-Cell Stage	Effective Date:	
		Date Reviewed:	24 October 2004
		Date Revised:	17 May 2006

This protocol was developed by the Monash University group [A.Trounson et al. (1987) Fertil. Steril., 48, 843-850]. Some modifications have been made.

Media Preparation

1. Cryoprotectant

- 1.1 Sucrose [Sigma S-7903] 860 mg
- 1.2 BSA [Sigma A-4378] 80 mg
- 1.3 PBS [Gibco 21600-069] 6.5 ml
- 1.4 DMSO [Sigma D-2650] 3.3 ml

2. Thawing media

- 2.1 Sucrose Solution 860 mg in 10 ml PBS
- 2.2 PBS
- 2.3 KSOM

3. FREEZING

- 3.1 Collect 8 cell stage embryos in a minimal volume of PBS.
- 3.2 Transfer embryos into the CPA inside the straw (30-40 µl CPA)
- 3.3 Heat seal the end of the straw.
- 3.4 Incubate straw on ice for 3 minutes.
- 3.5 Immerse the straw directly into LN2.

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

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