



<b>Protocol Section:</b>	<b>Embryo Cryopreservation</b>	<b>Policy No:</b>	P-CMMR1-ECD-00
<b>Protocol Subject:</b>	<b>Modified Ultra Rapid Embryo Freezing for ENU and Non-Enu Mice Protocol D</b>	<b>Effective Date:</b>	
		<b>Date Reviewed:</b>	24 October 2004
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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  $\mu$ l 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 LN<sub>2</sub>.

### 4. THAWING

- 4.1 Remove straw from liquid nitrogen.
- 4.2 Immediately immerse the straw in a 37 °C waterbath for 3-6 seconds
- 4.3 Remove the straw and dry with Kimwipe.
- 4.4 Cut off carefully the heat sealed end and expel the cryoprotectant solution into 2 ml of the sucrose solution.
- 4.5 Incubate for 10 minutes at room temperature.
- 4.6 Transfer embryos into a drop of PBS and incubate for 10 minutes at room temperature.
- 4.7 Wash the embryos very gently through several droplets of PBS.

**Issued by Lab Manager:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Approved by Facility Management:** \_\_\_\_\_ **Date:** \_\_\_\_\_