



# Protocols

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<b>Protocol Section:</b>	<b>Ovary Cryopreservation</b>	<b>Policy No:</b>	P-CMMR2-OC-01
<b>Protocol Subject:</b>	<b>Ovary Cryopreservation for ENU and Non-ENU Mice Collection, freezing and thawing procedures</b>	<b>Effective Date:</b>	
		<b>Date Reviewed:</b>	15 May 2003
		<b>Date Revised:</b>	<b>06 June 2006</b>

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## 1.0 Animals

- 1.1 Donor female mouse.
- 1.2 Recipient female mice (histocompatible with donor).
- 1.3 Breeding male mice.

## 2.0 Materials and Equipment

- 2.1 Controlled rate freezer (ThermoForma Cryomed 7452).
- 2.2 Stereo microscope.
- 2.3 Screw cap cryotubes (Falcon).
- 2.4 Disposable sterile plastic ware:
  - Filters, 0.22µm pore size
  - Pipettes
  - Petri dishes
  - 1ml syringe
- 2.5 Surgical instruments:
  - Two pairs of watchmakers forceps
  - Scissors
  - Serrefine clamp
  - Curved surgical needles (size 10)
  - Surgical silk suture (size 5-0)
  - Wound clips
- 2.6 Mouth pipette.
- 2.7 70% ethanol.
- 2.8 Forceps (large).
- 2.9 Tongs with long handles.
- 2.10 Liquid nitrogen container and boxes.
- 2.11 Permanent markers.

## 3.0 Reagents

- 3.1 Cryoprotectant (CPA): dimethylsulphoxide (DMSO).
- 3.2 Fetal bovine serum (FBS).
- 3.3 MEM (GIBCO).
- 3.4 Liquid nitrogen.
- 3.5 Anesthetic.

## 4.0 Methods

- 4.1 Collection of Ovaries

Donor mice are killed by cervical dislocation and their ovaries removed from the fat pad and the bursa aseptically. Removed ovaries are placed in a 10 X 35 mm disposable Petri dish containing 2 ml of MEM

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medium.

#### 4.2 Freezing

Transfer the ovaries from a single female into a cryotube containing 300 µl of cryoprotectant solution (CPA) [1.5 M dimethylsulphoxide (DMSO) in MEM medium supplemented with 10% fetal bovine serum].

Keep the sample in the CPA at 23 °C (room temperature) for 10 minutes and then place the cryotubes into the Programmed Freezer at 0 °C for approximately 30 minutes.

After the 30 minute equilibration, cool the cryotubes at a rate of 2 °C per minute to -7 °C and hold for 5 minutes.

Seed the cryotubes with a forceps that has been pre-cooled in liquid nitrogen. After seeding cryotubes are equilibrated at -7 °C for 5 minutes.

Start the controller to cool at a rate of - 0.3 °C per minute to -40 °C and, then at a rate of 10 °C per minute to -150 °C. At that point the cryotubes can be transferred to liquid nitrogen storage.

#### 4.3 Thawing

Thaw at room temperature (23 °C) for 40 seconds and then 30-35 °C water bath until ice is completely melted, and immediately after, remove the cryoprotectant and replace with 200 ml of medium. Allow the tissue to rehydrate in fresh medium for 10 minutes before surgery.

#### 4.4 Ovary Transplantation

Recipient females must be histocompatible with the ovary donor and anesthetized.

Aseptically, make a single transversal incision on the skin (hair removed) dorsally across the lumbar area to give access to the ovaries on both sides. Both host ovaries must be removed and a donor ovary is then transplanted orthotopically into the one or both side bursas. Recipients can be mated with a fertile adult male 7 days after the surgery.

### 5.0 Guidelines to customers

5.1 Alive animals are acceptable at the age of 3 weeks older. After that the younger the better.

5.2 The whole reproductive tracts are acceptable only if the tracts are put into PBS and kept at room temperature.

5.3 Dead animals are acceptable only if the animals are dead within 12 hours and kept in 4 °C refrigerator.

5.4 No contamination is accepted.

5.5 Call us for more information.

### 6.0 References

6.1 Candy CJ, Wood JM, and Whittingham DG. Restoration of a normal reproductive lifespan after grafting of cryopreserved mouse ovaries. Human Reproduction. 2000; 15 (6): 1300-1304

6.2 Szein, J.M., Sweet, H., Farley, J.S., Mobraaten, L.E. 1998. Cryopreservation and orthotopic  
 Canadian Mouse Mutant Repository

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transplantation of mouse ovaries: new approach in gamete banking. Biol. Reprod. 58:1071-1074.

## 7.0 Media Preparation

### 7.1 MEM preparation

Components	100 ml	200 ml	Molarity
EBSS 10x	10 ml	20 ml	1 x
NaHCO <sub>3</sub>	220 mg	440 mg	26.2 mM
Na Pyruvate	2.5 mg	5 mg	0.23 mM
Penicillin G	7.5 mg	15 mg	--
Streptomycin sulfate	5 mg	10 mg	--
L-Glutamine	29 mg	58 mg	2 mM
Essential Amino Acids AA 50x	2 ml	4 ml	1x
Vitamins 100x	1 ml	2 ml	1x
Phenol Red (11mg/ml)	0.1 ml	0.2 ml	--
EDTA	0.38 mg	0.75 mg	10 µM

Filter and store at 4 °C for no longer than 4 weeks.

Phenol red: 11 mg/ml.

Essential Amino Acids, 50x, Bio Whittaker, 13-606E

Vitamins, 100x, Bio Whittaker, 13-607C

EBSS	100 ml	200 ml	Molarity
CaCl <sub>2</sub> .2H <sub>2</sub> O	265 mg	530 mg	18 mM
KCl	400 mg	800 mg	53.6 mM
MgSO <sub>4</sub> .7H <sub>2</sub> O	200 mg	400 mg	8.11 mM
NaCl	6800 mg	13600 mg	1.16 M
NaH <sub>2</sub> PO <sub>4</sub>	125 mg	250 mg	10.4 mM
Glucose	1000 mg	2000 mg	55.5 mM

Calcium dihydrate should be dissolved first in a separate container with millipore H<sub>2</sub>O.

Add the CaCl<sub>2</sub> last after all the other chemicals have dissolved.

7.2 Freshly prepare 10 ml of 10% FBS in MEM by measuring 1.0 ml FBS into 9.0 ml MEM.

7.3 Freshly prepare 4 ml of 1.5 M DMSO by measuring 0.426 ml DMSO into 3.574 ml MEM supplemented with 10% FBS. Filter and use freshly.

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

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