8-Cell Slow Rate Cryo Thawing Method

1. Cryotubes are removed from liquid nitrogen storage and placed on the bench at ambient temperature until thawed, which usually requires from 12 to 15 minutes.

2. When completely thawed, 0.8 mL of modified Dulbecco's PBS is slowly added in a drop-wise fashion to dilute the DMSO, one drop every 5 seconds, swirling gently to facilitate mixing. (Slow addition is very important to prevent lysis of embryos.)

3. The contents of the cryotubes are gently withdrawn by means of a Selectapette (Clay Adams) and transferred to a dish where the embryos can be evaluated and collected.

4. Embryos are washed once in KSOM medium before putting into culture, or washed through drops of M2 for transfer to recipient females (day 0.5 pseudo-pregnant).

   If a programmed thaw is desired, it can be accomplished by removing the cryotubes from the liquid nitrogen refrigerator and placing them in the freezing chamber of a controlled-rate freezer which has been precooled to -100ûC and then warming them at 8ûC per minute until they reach +2ûC. The controlled warming rate is discontinued and the vials are allowed to warm to about +13ûC. The samples are allowed to liquify at ambient temperature for about two minutes before the DMSO is diluted out as described above.

III. Media

Phosphate Buffered Saline (Modified Dulbecco's)*

1. For a final volume of 100 ml of solution weigh out the following into approximately 75 ml of culture grade water:

NaCl	800.0 mg
KCl	20.0
KH2PO4	20.0
MgCl2.6H O	10.0
Dextrose	100.0
Na2HPO4	115.0
Sodium pyruvate	3.6
Penicillin G	7.5
Streptomycin sulfate	5.0

2. Dissolve 10.0 mg CaCl2 (anhydrous) in about 10 to 20 ml of culture grade water.

3. Add the dissolved CaCl2 to the above ingredients and bring the total volume up to 100 ml.

4. Use 20 ml of the above solution for making up a 2 M solution of DMSO (Aldrich).

5. Add 240.0 mg of crystallized Bovine Serum Albumin (BSA) and 0.1 ml of a 1% solution of Phenol red to the remaining 80 ml.
6. Sterilize the solutions by filtration.

*We have also used purchased dPBS 1X from Gibco, Cat. #14040-133, and added BSA to this.

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