Thawing embryos

This protocol may be used to thaw frozen mouse embryos either in straws or cryovials.

Keywords: JAX slow freeze | cryo straws | cryo vials | 8 cell mouse embryos

Expand

Reagents, supplies, and equipment:

- M2 medium (Millipore MR-015P-5D)
- KSOM medium (Millipore MR-020P-5D)
- paper clip for plunger
- scissors
- 35 mm culture dishes
- LN_2
- long forceps

Thaw embryos in straws:

Procedure:

1. Transfer the straw from the LN_2 storage freezer to a smaller container of LN_2.
2. Using forceps, grasp straw near the label and hold it in the air for 40 seconds, then submerge in room temperature water until the ice disappears.
3. Wipe straw dry.
4. Holding firmly, cut off seal and cut through PVA plug, leaving about half of the cotton plug in place to act as a plunger.
5. Using a metal rod, expel the entire liquid contents of the straw into a 35 mm culture dish. Do not let the plug drop into the dish.
6. Wait 5 minutes. The embryos will shrink considerably.
7. Transfer the embryos to a drop of M2. They will rapidly take up water and assume a normal appearance. Wait 5 minutes.
8. Wash the embryos in a fresh dish of M2 (wash through several drops of media if from a contaminated source - 10 times total) and then, either transfer to oviducts of a 0.5 dpc pseudopregnant mouse, or culture to blastocyst stage in KSOM and transfer to uterus of a 3 dpc recipient.

Thawing 8 Cell Embryos in Vials (Frozen using JAX slow freeze protocol):

1. Remove cryo tubes from LN_2 storage and place on the bench at room temperature until thawed, ~ 12-15 minutes.
2. When completely thawed, slowly add 0.8 ml of PBS to the cryo vial in a dropwise fashion to dilute the DMSO.
3. The contents of the cryo tube are withdrawn by means of a Selectapette (Clay Adams).
4. Wash embryos once in KSOM medium before putting into culture.*

*Additional washes (up to 10) may be necessary when cleaning up lines.