COSMO BIO CO., LTD.

1M DMSO and DAP213
(For cryopreservation of mouse oocytes)

Cat. No. CSR-R-T072 (DMSO)
CSR-R-T073 (DAP213)

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* Store at 4℃ until use. As a quality degrades after opening, use up all contents at one time.

A: Collection of oocytes

Collect oocytes of the objective development stage by *in vitro* fertilization or perfusion of oviduct after mating. (For Details, refer to instruction of HTF for *in vitro* fertilization and to KSOM or mWM for perfusion of oviduct)

B: Preparations

1. 1M DMSO is returned to room temperature before use.
2. Prepare cryotubes according to the number of germinal to freeze up.
3. Prepare cooling devices (such as crash ice, a chill heater or labtop cooler) to keep samples at 0℃ and chill DAP23 at 0℃ .
4. As cryotubes are stored in liquid nitrogen, prepare cryoboxes or cryocanes according to the numbers of tube.

C: Cryopreservation

1. Prepare drops of 1M DMSO (number of tubes +1) on the dish.
2. Transfer embryos to one of the drops and wait until they sink down.
3. After embryos sink down, divide them and transfer to rest of 1M DMSO drops into the numbers to be frozen with glass capillary.
4. Suck up embryos in 1M DMSO and transfer to a cryotube with micropipette (volume is adjusted 5μL).
5. Keep cryotube with embryos at 0℃ and stay for 5 minutes.

6. Add 45µL of DAP 213 to cryotube pouring to go along the wall of tube and stay for 5 minutes.
7. Transfer tubes to cryoboxes or cryocanes chilled with liquid nitrogen in advance and store them soaking in fluid phase of liquid nitrogen.
8. Preservation is done in fluid phase of liquid nitrogen. If tubes are located in air stratum, samples may melt and survival rate after reconstitution will be severely be decreased.

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