

Shipping Refrigerated Embryos

1.0 Equipment

- 1.1** Dissecting instruments (fine-pointed scissors, fine forceps)
- 1.2** Refrigerator (4-8°C)
- 1.3** Incubator (30°C, 5% CO₂)
- 1.4** Embryo handling device
- 1.5** LN₂ carrying dewar

2.0 Supplies

- 2.1** Lifor + Quercetin + DMSO
- 2.2** 70% alcohol
- 2.3** Parafilm
- 2.4** Sellotape
- 2.5** Cold transportation kit inc.
- 2.6** Aluminium box
- 2.7** Gel cool packs
- 2.8** Biotube
- 2.9** Polystyrene container
- 2.10** Packing tape
- 2.11** 0.5ml PCR tube
- 2.12** Tips for embryo handling device
- 2.13** LN₂
- 2.14** Tissue
- 2.15** Metal rod

- 2.16** Scissors
- 2.17** Embryo culture dishes (3004)
- 2.18** KSOM
- 2.19** M2 media

3.0 Procedure

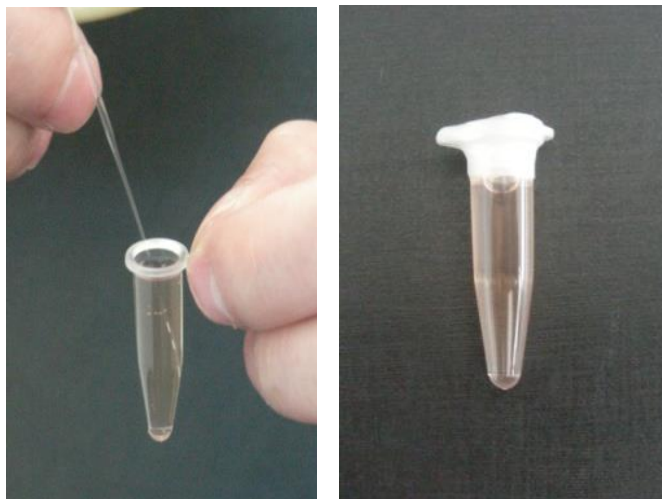
3.1 Thawing embryos

- 3.1.1 Thaw the embryos following an appropriate protocol.
- 3.1.2 Transfer embryos into a 200 μ l drop of KSOM, overlaid with silicone fluid and culture for 2-3hrs in a CO₂ incubator at 37°C.

3.2 Preparation of embryos for shipment

- 3.2.1 Make 3 x 150 μ l M2 drops in a culture dish.
- 3.2.2 After the embryos have been cultured in the incubator wash them through the 3 drops of M2.
- 3.2.3 Fill 0.5ml PCR tube to the top with 0.6ml M2 medium at room temperature, then load 30-40 embryos into each PCR tube and seal with parafilm (Picture 1).

Picture 1



3.3 Preparing the refrigerated package

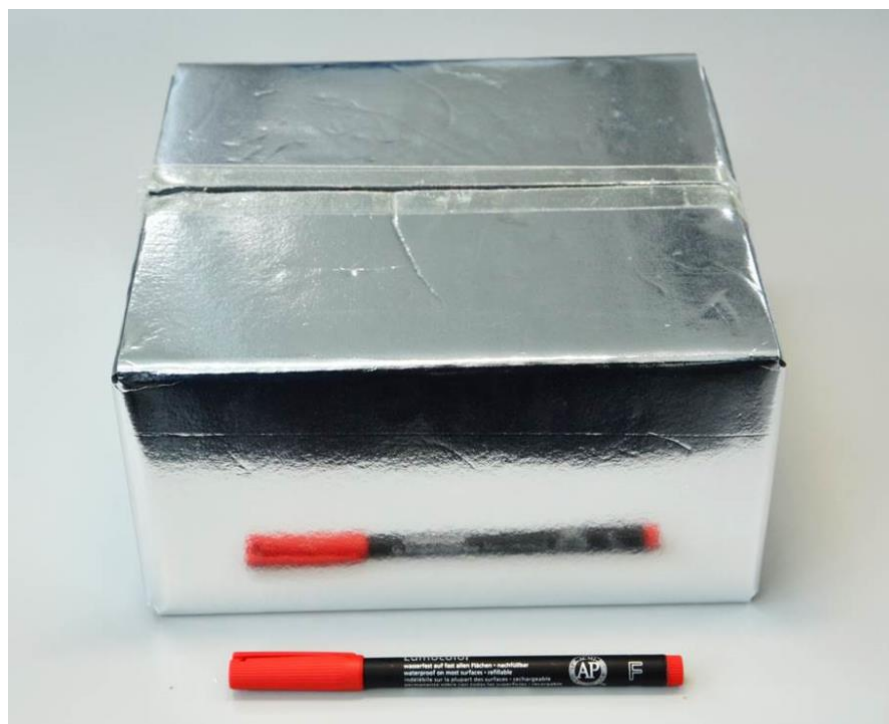
- 3.3.1 Cool the aluminium lined box, and the contents, in the refrigerator for 48hrs prior to the shipment.
- 3.3.2 Place the tube containing the embryos into a biotube which is supplied within the cold transportation kit (Picture 2).

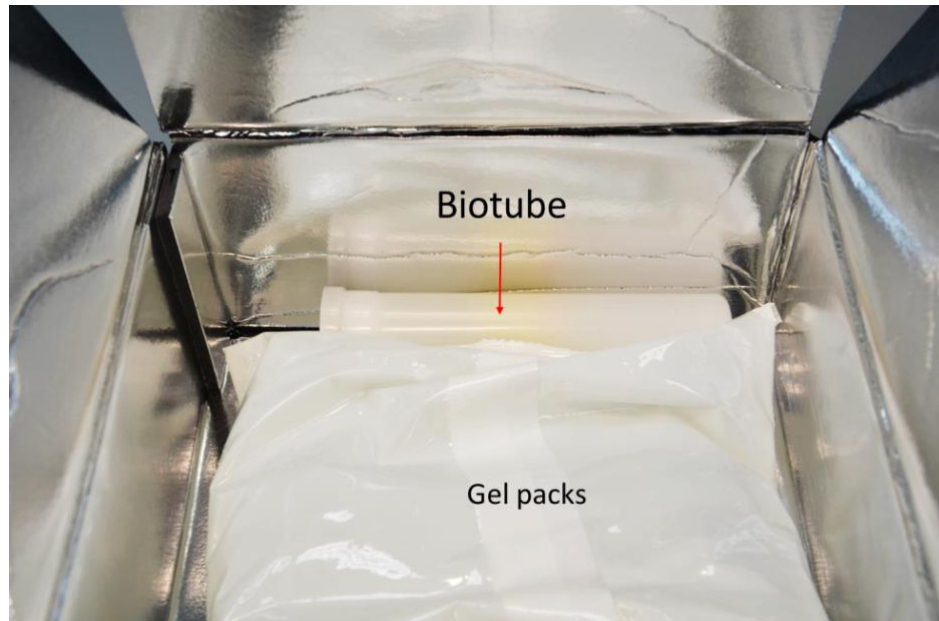
Picture 2



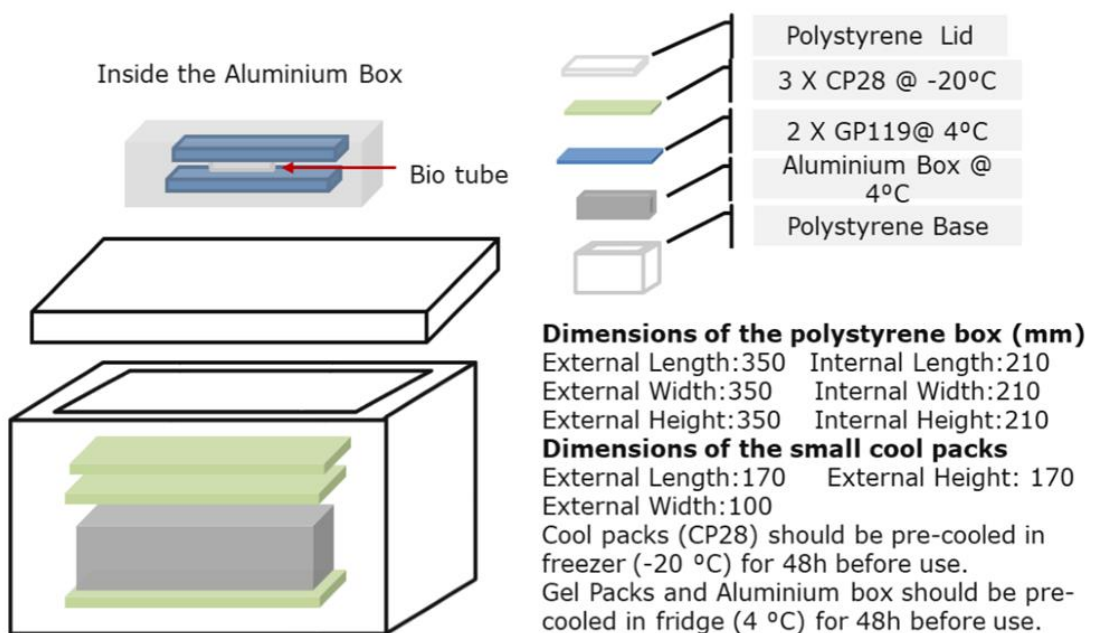
- 3.3.3 Place the biotube into the aluminium lined box (Picture 3), then place two gel cool packs into the lined box, so they surround the biotube (Picture 4).

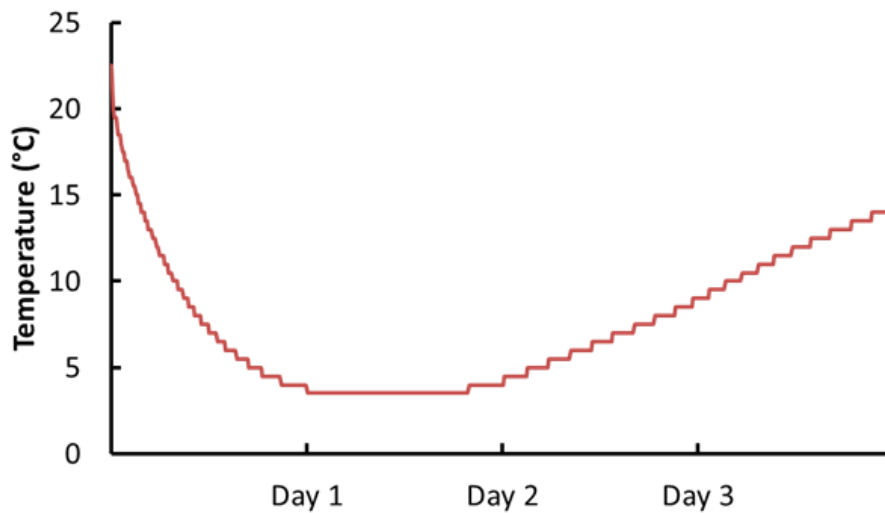
Picture 3



Picture 4

- 3.3.4 Seal the aluminium box with sellotape.
- 3.3.5 Place the aluminium box into the polystyrene container following the assembly instructions. Then seal the polystyrene container with packing tape (Picture 5). This thermal control unit will maintain a temperature of 4-8°C for up to 72hrs (Picture 6). The embryos will be viable for at least 72hrs under these conditions.

Picture 5

Picture 6

3.3.6 Send the samples via a regular delivery service.

3.4 Arrival of the refrigerated package

- 3.4.1 When the cold package arrives, remove biotube from the aluminium box and remove the PCR tube containing embryos from the biotube.
- 3.4.2 Allow tube to stand vertically at room temperature for 30mins avoiding direct exposure to light.
- 3.4.3 Open the PCR tube containing the embryos and gently re-suspend the embryos.
- 3.4.4 Aspirate the entire M2 medium from the tube using 1000µl pipette and then transfer the solution to the centre of an embryo culture dish.
- 3.4.5 Locate the embryos and transfer them to a 200µl drop of fresh M2 medium.
- 3.4.6 The embryos are now ready for use and may be transferred into recipient females or cultured in KSOM overlaid with silicone fluid, until required.