University of Basel

Centre for Transgenic Models
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CTM

EMBRYO THAWING PROTOCOL

Material

Before beginning, make sure that all equipment is at hand so that steps 1 to 5 can be performed as rapidly as possible!

Thawing Media (0.5 M Sucrose)

- Sucrose 1.71 g
- M2 medium with Pen/Strep added to 10ml

Prepare freshly and filter through a 0.22 µm Millipore filter.

Thawing

1. Remove straw from liquid nitrogen using tweezers
2. Wait a few seconds for the liquid nitrogen to be expelled from the straw. Face the open end of the straw away from you at this time
3. Rapidly immerse the end of the straw in a 37°C water bath so that the section containing cryoprotectant is completely submerged and leave for approx. 3 seconds.
   Cryoprotectant should remain clear.
4. Remove the straw and rapidly wipe dry with a Kimwipe
5. Cut off the heat-sealed end and expel the cryoprotectant solution into 3 ml of the 0.5M sucrose solution in a 6cm tissue culture dish
6. Incubate for 5 minutes at RT without shaking or mechanical disturbance.
7. Transfer embryos into a second dish with 3 ml M2 medium and incubate for 5 minutes at RT undisturbed.
8. Wash the embryos very gently through several droplets of M2.
9. Transfer the embryos into the oviduct of recipient mice using M2 as transfer media.

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