

Cryopreservation of material for thick sectioning

Protocol author: Virginia Weis

Date: 2001

Materials needed

1. Narcotizing agent – e.g. .37M MgCl₂ diluted 1:1 with seawater
2. TFM – tissue freezing medium (Fisher cat # 15-183-13 about \$12)
3. Little wells – that hold approx 1 ml – ie a porcelain dish with multiple wells in it
4. Small spatula or forceps
5. Razor blades or 2” diameter petri dishes
6. Dessicant

Procedure

1. Relax animals in a small volume of narcotizing agent for a few minutes
2. Squirt a dollop, around 0.5 – 1 ml, of TFM into each of three wells in dish. Avoid introducing air bubbles in the dollop.
3. Pick up animal with forceps or spatula and move to 1st TFM well. Try to minimize the amount of water you transfer with your animal.
4. Gently swirl animal in TFM, being careful to minimize introduction of air bubbles as you swirl.
5. Transfer animal to next dollop and swirl and then to next and swirl.
6. Prepare a fairly small drop – 3 – 5 mm in diameter - of fresh TFM on the razor blade or in 2” petri dish.
7. Move animal to drop- minimizing amount of TFM you transfer with animal and minimizing introduction of air bubbles.
8. Freeze immediately in super cold in a box with dessicant