

Making up 20x and 1x embryo medium

Equipment:

1. 2L glass bottle
2. Salts NaCl, KCl, CaCl-2H₂O, KH₂PO₄, NaHPO₄ anhydrous, MgSO₄-7H₂O
3. Distilled water

Procedure: for 20x

1) Weigh Out:

17.5g NaCl

0.75g KCl

2.9g CaCl-2 H₂O

add distilled water to 800mls

0.41g KH₂PO₄

0.142g Na₂HPO₄ anhydrous

4.9 MgSO₄-7H₂O

add distilled water up to 1 L and mix if necessary using stirrer. Store at 4 C.

Working solution 1x embryo medium

- 1) 50 ml of 20 x embryos medium
- 2) 2 ml 500 x NaHCO₃ (0.35g in 10 ml Distilled H₂O)
- 3) Fill to 1 L with distilled water. Store at room temperature and make fresh every week.

Making up 50ppm Povidone Iodine and 25ppm working solution. 2016

Equipment:

1. Povidone Iodine
2. Embryo medium (see embryo medium protocol)
3. 250ml Amber glass bottles.

Procedure:

- 2) Weigh out 0.036g of povidone iodine
- 3) Place into 250 ml amber bottle
- 4) Fill bottle with 250 ml of 1 x embryo medium.
- 5) This is the stock 50ppm of iodine.

Working solution 25ppm

- 1) Take 50 ml of stock 50ppm.
- 2) Place into flask, add 50 ml of 1x embryo medium making 25ppm working solution for bleaching.

Making up Artificial salt water.

Equipment:

4. 1L bottle
5. Instant ocean salts
6. Distilled water

Procedure: Stock salts

1. Weight 40g Salt
2. Add to 1 L bottle
3. Add distilled water to 1 L. Mix as needed

Working solution Egg water:

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| <ol style="list-style-type: none">4) 1.5ml of stock salts5) Add to 1L of distilled water. |
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Disinfecting Protocol using Iodine 2016

Equipment:

4. 5 x petri dishes
5. Sieve
6. 1 x Embryo medium (see Embryo medium protocol)
7. 25ppm Iodine solution (see Iodine protocol)
8. Fish water (see Artificial salt water protocol)
9. Timer
10. Plastic 3ml pipette

Procedure:

- 1.) Put out the petri dishes in a line and fill one with 25ppm Iodine and 3 with Artificial water. And final one with Embryo medium to collect embryos in at the end.
- 2.) Place embryos into the sieve using pipette. Then pass the sieve with embryos into each of the petri dishes for the following times and order:
 - a) 2 minutes in Iodine solution dish
 - b) 1 minute in 1st dish artificial water
 - c) 5 seconds rinse in 2nd dish artificial water
 - d) 5 seconds rinse in 3rd dish artificial water
- 3.) Transfer embryos into final petri dish with embryo medium and place into clean incubator before they go to the new room.