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:

4) 1.5ml of stock salts
5) Add to 1L of distilled water.
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.