Preparation of Embryonic Extract

Materials

1. Ice on tray.

2. Dissection tools: 1 pair of large scissors; 2 pairs of fine scissors; 1 pair of coarse forceps; 2 pairs of fine forceps; soak in 95% EtOH.

3. 70% EtOH in a spray bottle.

4. 2 sets of 100 mm sterile petri dishes (glass or plastic) on ice.

5. 1x 50 ml sterile beaker, keep on ice.

6. 3x 250 ml sterile beakers, keep on ice.

7. Cheese cloth, sterilized by autoclaving.

8. 250 ml Saline G, on ice.

9. 25-50 ml Hank's balanced-salt solution, on ice.

10. 12-day chicken embryos, 12.

11. Polytron with PTA 10TS generator or a Dounce homogenizer with a Teflon pestle of coarse fitting.

12. 50 ml conical disposable centrifuge tubes.

13. 50.2 Ti rotor and tubes, chilled.

14. Millipore filters and holder: type AP, AW, and AA, 47 mm diameter.

15. Cameo 0.22 um filters (Fisher Scientific).

16. 50 ml syringe for applying pressure.

17. 3x 25 ml sterile flasks.

18. 15 ml conical disposable centrifuge tubes.

Procedure (perform in a still air hood)

1. Spray eggs twice with 70% EtOH.

2. Wipe eggs with 95% EtOH thouroughly, let dry completely.

3. Collect 9-12 embryos in a sterile 100 mm dish on ice, use large scissors, coarse forceps and the first pair of fine forceps.

4. Cut off embryo heads and discard, use 1st fine scissors.

5. Slit embryos ventrally, pierce heart 3-4x to drain blood, use 1st pair of fine scissors. Cut legs off and discard.

6. Transfer embryos to the second sterile 100 mm pertri dish on ice, use second pair of forceps. Clean up the first dish with Kimwipe.

7. Chop each embryo 4-5x with the second pair of fine scissors.

8. Divide saline G (250 ml) into 3x 250 ml beakers (~75 ml each).

9. Transfer chopped embryos to a square piece of cheese cloth, spread on the first petri dish.

10. Transfer embryos wrapped in cheesecloth from one beaker to the next to wash the embryos. After the last dip, squeeze with a forcep against the side of the beaker to drain liquid.

11. Transfer the tissue into a 50 ml beaker - keep on ice. Use a forcep for solid pieces.

12. Homogenize with the Brinkmann Polytron (Brinkmann PTA 10TS generator), until indivicual pieces are no longer visible. Keep the beaker on ice. Alternatively, a Dounce homogenizer with coarse fitting can be used. Homogenize for 10-15 strokes.

13. Transfer no more than 25 ml of the homogenate to a sterile 50 ml conical centrifuge tube. Read volume.

14. Dilute 1:1 with Hank's balanced-salt solution. Use the Hank's solution to rinse the Dounce before adding to the tube. Mix the content in the tube by shaking.

15. Freeze in -70oC freezer. Purpose of freeze-thaw cycle is to lyse cells. The suspension may be left overnight in the -7O^{o}C freezer if desired.

16. Thaw in cool H2O.

17. Repeat steps 15 and 16.

18. Spin in a 50.2 Ti rotor, 33,000 rpm, for 1 hour at 4°C.

19. Collect supernatant immediately into a test tube. Some turbid material near the top of the supernatant may be included.

20. Assemble filters. Filter no more than 25 ml through prefilters, in the following manner: 1x through Millipore thick prefilter type AP; 1x through Millipore thin prefilter type AW; 2-4x through Millipore 0.8um filter type AA. Handle filters with forceps. Collect filtrate in 50 ml chilled flasks. Open the holder over the flask to collect residual fluid. Assemble all filters before starting the filtration.

21. Sterile filter through a Cameo IV 0.22um filter, ~10 ml per filter. Collect filtrate in two 15 ml conical tubes.

22. Store in -20°C freezer (usually good for about 2 weeks).