

CHICKEN EMBRYO FIBROBLASTS

makes 50 plates from 12 eggs

Solutions:

500 ml Trypsin (1x in PBS)
500 ml PBS
2 x 500 ml Ham's F-10 + 10% FCS + 1% Chicken Serum
70% EtOH
100 ml Freezing media (25% FCS, 8% DMSO in Ham's F-10)

Equipment:

2-200 ml beakers autoclaved with stirbars
2-150mm Plates
500 ml beaker
stir plate
dissection tools (2 tweezers, 1 pair scissors)
50 plates P-100
2 corning 50 ml centrifuge tubes
500 ml beaker of Tupperware container of same size
sterile slide and razor blade.

Procedure:

- 1) On day 11-12 candle 1 dozen fertile white leghorn eggs. Mark abnormalities on egg.
On day 12, warm Trypsin, 1 bottle media, and 2 bowls of water.
Place in hood (w/ UV on) beakers, 150 mm plates, dissection tools in EtOH.
- 2) In hood, half fill one of the plates w/PBS. Place 50ml beaker in the other half, place 50 ml of Trypsin in both 200 ml beakers. Sterilize eggs with 70% EtOH.
Place egg in beaker pointed end down.
- 3) Crack open wide end. Remove shell.
- 4) Pull out embryo. Rinse by pouring PBS over embryo over 500 ml beaker.
- 5) Put embryo into dry plate. Remove head close to body, limbs, and internal organs (heart, lungs, liver, kidney).
Place embryo into plate with PBS to rinse.
- 6) Mince on slide with sterile razor blade. Slide tissue into beaker of trypsin.
Collect 5-6 embryos in 1 beaker of trypsin.
- 7) Stir at medium speed in 37°C bowl of water about 5 min.
- 8) Spin down cell debris at #2 for 10 min.
- 9) Pour supernatant (containing cells) into a new corning tube. Spin at #5 for 5 min.
- 10) Resuspend cell debris in about 40 ml trypsin and return to beaker with stirbar. Continue stirring as before for 5 min. Repeat spins pooling cells each time.
- 11) When all the cells are pelleted, resuspend in 10ml PBS and break up clumps.
Spin @ #5 for 5 min.
- 12) Bring pellet up in 50 ml Media. Place 1 ml of cells into 50 plates each with 7 ml media.
Place in incubator.

Next day: Rinse cells with PBS to remove floaters and add fresh media.

Following day: Trypsinize plates, pool cells in 2 corning tubes.

Add 10 ml Media to inactivate trypsin.

Spin cells down #5 for 5 min.

Resuspend each tube in 50 ml Freezing media.

Aliquot out 1 ml into each freezing tube.

Freeze in -80°C .

If cells are to be maintained, passage with no more dilution than 3 or 5 (i.e., 1 plate split into 3-5 new plates.)