Chick or mouse embryo whole mount immunohistochemistry

Procedure based on protocols and information kindly provided by:

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The following procedure describes the procedure for whole mount staining of chick or mouse embryo's. A similar procedure could be used for staining of drosophila embryos. Incubations for fixative, blocking buffer, antibody, wash buffer, permeabilization and substrate color development will need to be much longer than normal immunocytochemistry / immunohistochemistry to allow for permeabilization right into the centre of the sample.

Procedure:

1. Obtaining the embryo:

Chick: Gently break the egg into a medium sized clean glass petridish. The embryo will naturally float to the top of the yolk. It will then be visible for careful removal using clean scissors and a Pasteur pipette with the tip removed (this prevents any damage to the embryo from the narrow end of the pipette).

Mouse: Operate on adult female to remove embryos.

Dissect the embryo in ice cold PBS removing as much unwanted tissue as possible.

We recommend to remove as much embryonic membrane and excess tissue as possible as this can prevent the antibody perfusing into the embryo.

2. Place embryo in a 5 ml bijous in 4% paraformaldehyde. Leave to fix 4°C. The time required will need optimization. We suggest trying between 2 hours and overnight.

OR fix in m-DMSO (80% methanol, 20% DMSO) or other fixative of choice.

Generally whichever fixative has been used successfully with the antibody when used in cryosections, this fixative should be suitable for whole mount. However, this may require some optimization.

When the sample is fully equilibrated with the fixative (i.e the fixative has permeabilized the whole sample) then it should sink to the bottom of the solution. Ensure the sample has sunk to the bottom of the fixative before proceeding.

3. Wash 3X in PBS 0.5 - 1% Triton thirty minutes each time.

4. Incubate the embryos twice for 1 hr in block (PBS 1% Triton + 10% FCS + 0.2% Sodium Azide), room temperature.

5. Incubate embryos in peroxidase block (0.1% H_2O_2 diluted in blocking buffer) overnight 4°C.

6. Wash embryos 2X in blocking buffer

7. Transfer embryos using Pasteur pipette with the end cut off to a 2 ml tube. Add primary antibody at the required dilution / concentration.

It is recommended that as incubations can be very long in whole mount staining, the antibody should be diluted in blocking buffer containing 0.02% sodium azide to prevent microbial growth.

8. Incubate for 1 to 4 days on a gentle rotation devise at 4°C.

This incubation time will require some optimization depending on the antibody and also the size of the embryo.

9. Wash embryo's 3X 1 hr in PBS 1% Triton + 10% FCS 0.2% sodium azide

10. Wash 3X 10 minutes in PBS 1% Triton

Wash well to remove traces of sodium azide as this will inhibit peroxidase activity when developing

11. Add secondary antibody in blocking buffer (no sodium azide)

12. Inducate for 2 to 4 days with gentle rotation 4°C

13. Wash 3X 10 minutes in PBS 1% triton

14. Incubate embryos in DAB substrate for 2 to 3 hrs RT.

15. Transfer embryos to a dish and add fresh DAB plus 5 µl H_2O_2 per 1 ml of DAB.

16. Rinse three times in PBS once reaction and staining have reached desired intensity

17. Mount and view embryo's. Store at 4°C until analysis.

Mounting:

1. Place sample in 100% glycerol for 48 hours. When sample is fully equilibrated with the glycerol (i.e it is fully perfused with the glycerol) it will sink to the bottom of the vial. Ensure the sample is at this stage before proceeding.

2. 75% glycerol has approximately the same density as gelatin which is used to mount and set the samples on a slide. Therefore, samples should be equilibrated in 75% glycerol after staining for approximately 15 minutes (again, when equilibrated, the sample should sink).

3. Place in 50% glycerol until the sample sinks. The embryo can be imaged at this stage, or mounted whole in the glycerol on a slide. Use grease around the edges of the coverslip for protection.

If the sample is to be embedded in gelatin and sectioned on a vibratome; place 20% gelatin pre-warmed to 65°C. Leave for approximately 30 minutes to equilibrate before taking out the sample to mount. When equilibrated in the gelatin, the sample should sink to the bottom.