

Immunofluorescence Staining of Embryos

Day One:

1. Transfer embryos to 2ml screw cap tube in 100% EtOH.
2. Rehydrate embryos through 50-70% EtOH and 30% EtOH, 5 minutes/wash
3. Wash 2 x 5 min. in PTW (PBS + 0.1% Tween-20)
4. Block for 2 hr. at room temp. in PTW + 10% HIGS
5. Incubate o/n at 4°C in 1° Ab diluted in PTW + 10% HIGS

Day Two:

1. Wash all day at room temp in PTW with several changes of PTW.
2. Add 2° Ab diluted in PTW + 10% HIGS, incubate o/n @ 4°C.

Day Three:

1. Wash embryos in PTW at room temp with several changes for as long as possible.
2. Prepare slides for mounting the embryos-glue square coverslips to the edge of a slide or the edge of a large coverslip leaving a "well" in the middle.
3. Add a drop of PVA mounting medium to the middle of the well and transfer embryos into the PVA-avoid transferring PTW if possible.
4. Place a coverslip over the embryo-glue the coverslip in place.
5. View on confocal or other IF scope.

Notes:

-Staining will work with 2 hour antibody incubations and several washes between antibodies.