

Preparing injected embryos for immunofluorescence analysis

(As in Papoulas, et al. (2005) Nature Cell Biology 7:612-618.)

1. Use a single-edge razor blade to cut away as much halocarbon oil as possible without disrupting or exposing the injected embryos.
2. Carefully wash the excess halocarbon oil away into a glass waste container using heptane delivered from a 9" Pasteur pipet. Once you have begun to expose the embryos, hold the glass slide or cover slip over a glass petri dish and vigorously wash the embryos off of the heptane glue and into the glass petri dish. Tilt the glass petri dish slightly in order to collect the embryos against the wall.
3. Use a 9" Pasteur pipet to transfer the embryos into a small glass vial. It is critical that the embryos remain in the neck of the Pasteur pipet, if they enter the wide portion of the pipet they will stick to the glass and be irretrievable.
4. Once the embryos have been transferred to a vial, remove as much of the heptane as possible without exposing the embryos. Add 0.5 mL-1.0 mL fresh heptane followed by an equal volume of fixative and fix for the appropriate time (e.g., 0.5 mL-1.0 mL 37.5% formaldehyde for 5 min.).
5. Using a 9" Pasteur pipet transfer the embryos at the heptane-fixative interface onto a homemade embryo collection basket. Again, keep the embryos in the neck of the pipet. The collection basket can be made from a 50 mL plastic tube and Nytex mesh. Cut off the top 1" of a 50 mL tube containing the threads and cut out the center of the cap to make a ring that screws onto the tube. Place the mesh over the threaded end of the tube and secure the mesh by screwing the cap ring over the mesh and tube. It is essential that the Nytex is soft and that it forms a cup while held by the rings. Place the collection basket on the rim of a wide-mouth glass bottle or flask. Once all the embryos are collected on the Nytex mesh rinse them thoroughly with heptane. It is essential to keep all of the embryos in one area of the Nytex.
6. Carefully disassemble the collection basket and wick away the excess heptane by pressing several Kimwipes against the underside of the Nytex mesh. Quickly, but gently, transfer the embryos from the Nytex mesh onto a piece of double-stick tape in the bottom of a 3.5 cm plastic petri dish by lightly pressing the embryos onto the tape. Once the embryos have been transferred to the tape immediately pour a small volume of 1X PBTA onto the embryos to prevent their dehydration. Step 6 is the most difficult part of the procedure for a couple reasons. First, if not enough heptane is wicked away from the Nytex the embryos will not stick to the tape. If too much is wicked away you will lose good embryo morphology. Second, if you do not press the embryos onto the tape firmly enough they will not stick, yet if you press them too firmly they will flatten and good morphology will be lost.
7. Under a dissecting microscope hand devitellinize the embryos with the point of a 23 gauge needle attached to a 5 mL syringe. Gently tap embryos at either their anterior or posterior ends with the needle until they pop free of their vitelline shell and the heptane glue.
8. Finally, transfer the devitellinized embryos to a 1.5 mL eppi tube using a 9" Pasteur pipet and prepare them for immunofluorescence analysis. Again, keep the embryos in the neck of the pipet.