

# Fixing Zebrafish for Histopathology

## A. Summary

Post-mortem autolysis and putrefaction (post-mortem decomposition caused by non-pathogenic bacteria) occurs rapidly once a fish dies. This makes fish that are found dead poor specimens for histopathology. For optimum preservation and diagnostic value, fish must be alive and euthanized just prior to fixation. Fish designated for histopathology should be euthanized according the Euthanizing fish for PCR or Fixation SOP.

## B. Definitions

N/A

## C. Materials

1. Supplies for euthanasia (see Euthanasia of Fish for PCR or Fixation)
2. Euthanasia of Fish for PCR or Fixation SOP
3. Fine forceps
4. Dissecting or micro-dissecting scissors
5. Plastic spoon
6. Paper towel or absorbent surface
7. Falcon tubes or other sealable containers for holding fish in fixative.
8. Tape and permanent pen to label tubes
9. Parafilm
10. Test tube rocker or nutating mixer
11. Dietrich's fixative (~10 mL per adult fish)

The following recipe makes 100mL of fixative. Prepare and dispense Dietrich's fixative in a laboratory fume hood. Store at room temperature.

- 30 ml Ethanol (95%)
- 10ml Formalin (Formaldehyde 37% solution, histological grade, contains 10-15% methanol)
- 2 ml Glacial Acetic Acid
- 58 ml Distilled water

## D. Procedures

1. Euthanize fish according to Euthanasia of Fish for PCR or Fixation SOP.
2. Use the plastic spoon to remove the fish from the ice bath, tilting the spoon against the side of the crossing cage to drain off the water. Place the fish on its side on the paper towel.
3. Use the scissors to cut off the tail just forward of the tail fin, through tail muscle but behind the anal fin. The tail should be left intact if there is a lesion on the tail or if the fish is less than 1.5 cm in length.
4. Using the fine forceps, pull the skin over the belly up, to avoid disrupting the internal organs, and make a small cut in the raised skin with the scissors. This step may be skipped if the fish is very skinny or less than 1 cm in length.
5. Use the forceps to pick the fish up by a fin and place it in a sealable container of fixative. The volume of fixative should be at least ten times the volume of the specimen. 10 ml of fixative is sufficient for the average sized adult zebrafish.
6. Tighten the lid and cover the container-lid interface with Parafilm.
7. Use the tape and permanent pen to label the container with the fish stock number, tank of origin, allele, fertilization date, and, if present, clinical signs of disease.
8. Place the container on a test tube rocker or nutating mixer at low speed for at least 24 hours.
9. Fish may be stored in Dietrich's fixative for several weeks if necessary.

## E. Monitoring Requirements

N/A

## F. Record Management

Samples fixed for histopathology will be assigned a pathology case number and fish number. Fish identification information and histopathology results will be recorded in the pathology database, which is collated by case number.

## G. References

N/A

## H. Other SOPs Cross-Referenced Here

Euthanasia of Fish for PCR or Fixation SOP

## I. Supplementary Documents

N/A