A Simple Whole-Mount Staining Protocol For Bone And Or Cartilage In Adults And Larvae

(Source: T.A. Franz-Odendaal from Zebrafish Book 5th Edition)

This protocol was originally modified from Klymkowski and Hanken (1991) for amphibians. Although you can stain both cartilage and bone in the same specimen, you need to be aware that bone staining can mask cartilage staining, for instance when perichondral ossification takes place around a cartilage template. It is therefore better to stain bone and cartilage in different specimens of the same developmental stage.

Small plastic baskets with a mesh bottom are ideal to hold the specimen while staining and for transferring the specimen to the next solution. In the latter part of each protocol, the specimen becomes very delicate and can be damaged easily. All steps are conducted at room temperature.

**Whole-mount staining for bone**

Fix the fish in 10% neutral buffered formalin for a few hours to one day depending on the size of the specimen.

Rinse the specimen in tap water overnight.

Eviscerate the specimen removing internal organs being careful not to damage the ribs. Skin the fish if possible.

Place the specimen in 1% KOH with 3% hydrogen peroxide (about 5 ml 3% H2O2 in 100 ml 1% KOH solution) until the pigment is bleached. Black pigment should be brown to light brown. Do not over do this step because some tissues, especially eyes, can burst. Usually this step takes one day for 15-20 mm long fish, but can take longer.

Rinse the specimen in tap water for 30 minutes.

Transfer the specimen to 30 ml saturated sodium tetraborate in 70 ml of H2O for 12 hours or overnight.

Place the specimen in 1% KOH with 1mg/ml Alizarin Red overnight. The solution should be a deep purple color.

Rinse the specimen in tap water for 30 minutes.

Place the specimen in 1% trypsin in 2% borax overnight or until cleared. This step is complete when 85% of the soft tissue is dissolved. This step may take several days. Very little further clearing will take place in the steps following this one. For a 15-20 mm long fish, one to two days is usually sufficient.

Transfer the specimen through a series of 1% KOH/glycerol solutions. The specimen can be transferred when the specimen sinks. Overnight for each solution is typical.

20 ml 100% glycerol + 80 ml 1% KOH
40 ml 100% glycerol + 60 ml 1% KOH

Transfer the specimen to a glycerol/alcohol mixture for long-term storage (70ml 100% glycerol + 30 ml 70% alcohol).

**Whole-mount staining for cartilage**

Fix the fish in 10% neutral buffered formalin for a few hours to one day depending on the size of the specimen.

Rinse the specimen in tap water overnight.

Eviscerate the specimen removing internal organs being careful not to damage the ribs. Skin the fish if possible.

Rinse the specimen for 30 minutes in tap water.

Place the specimen in an acetic acid/alcohol/alcian blue solution overnight (20 ml acetic acid + 80 ml 100% alcohol + 15 mg Alcian blue).

Rehydrate the specimen through a graded series of alcohols (100% alcohol, 80% alcohol, 60% alcohol, 40% alcohol, 20% alcohol) to water. 1 hour in each solution.

Place the specimen in 1% trypsin in 2% borax overnight or until cleared. This step is complete when 90% of the soft tissue is dissolved. This step may take several days. Very little further clearing will take place in the following steps. For a 15-20 mm long fish, one to two days is usually sufficient.

Bleach the specimen in 1% KOH with 3% hydrogen peroxide as described above (step 4 of the whole-mount bone protocol).

Transfer the specimen through a graded series of 1% KOH to 100% glycerol solutions as described above (step 10 of the whole-mount bone protocol).

Store the specimen in 100% glycerol.

**Reference**


**Acknowledgement**

This protocol was developed while working in B.K. Hall’s laboratory (Dalhousie University, Canada) and after discussions with P.E. Witten (The Institute of Aquaculture Research in Sunndalsøra, Norway).