Adult No Acid Bone & Cartilage Stain

For fish 40 mm Standard Length or over.

Protocol

All steps except last on rotator

- Fix: 2% PFA overnight @ room temperature
- Wash: Tris/MgCl₂; 1 hr
- Stain: 0.01% Alcian Blue in 10 mM MgCl₂, pH 7.5; overnight @ room temperature
- Differentiate/Re-hydrate: 80% EtOH/100 mM Tris, pH 7.5/10 mM MgCl₂; 30 min or more

50% EtOH/100 mM Tris, pH 7.5; 30 min or more

25% EtOH/100 mM Tris, pH 7.5; 30 min or more

0.5% KOH; 30 min or more

- Bleach: 3% H₂O₂, 0.5% KOH until pigment is almost gone in body & eyes are light brown; usually several hours
- Digest/clear: 35% NaBO₃ 30 min or more 1% Trypsin in 35% NaBO₃ depending on the size of the fish; several hours to overnight @ room temperature or @37°C to speed up the process or @4°C to slow down the process. Lower speed of rotator after this step.
- Wash: 10% glycerol/0.5% KOH; 1 hr or more
- Stain: 0.02% Alizarin, pH 7.5; overnight @ room temperature
- Differentiate: 50% glycerol/0.5% KOH; 1 quick wash, 30 min or more;

2nd wash on rotator overnight or more.
- Store: without agitation 4°C @ room temperature
- Long term storage: 70%-100% glycerol (in H₂O, not KOH) with a couple of thymol crystals; @ room temperature

Solutions for adult fish:

2% PFA/1XPBS pH 7.5:

per ml:

250ul 8% PFA
100ul 10XPBS
650ul water

100 mM Tris pH 7.5/25 mM MgCl₂

To make 50 ml:

5ml 1M Tris pH 7.5
1.25ml 1 M MgCl₂
43.75ml water

0.2% Alcian Blue/90%EtOH Stock: Alcian Blue will not readily dissolve in EtOH.

To make 100 ml: Add 0.2g Alcian Blue 8GX powder (Anatech, Ltd. #862) to 11.2ml 50% EtOH. Warm and occasionally swirl. When all is dissolved, add 95% EtOH to 100 ml. Check clarity of solution under a microscope to make sure there are no precipitates.

0.01% Alcian/25 mM MgCl₂ Stain pH 7.5

To make 50 ml:

2.5 ml 0.2% Alcian Blue 8GX in 90% ETOH
5ml 1M Tris pH 7.5
1.25ml 1M MgCl₂
37.6ml 95% ETOH
8.65ml water
Ethanol rehydrations:
To make 50 mls:

80% ETOH/25mM MgCl$_2$
- 42.1ml 95% ETOH
- 5ml 1 M Tris pH 7.5
- 1.25ml 1M MgCl$_2$
- 1.65ml water

50% ETOH:
- 26.3ml 95% ETOH
- 5ml 1M Tris pH 7.5
- 18.7ml water

25% ETOH:
- 13.2ml 95% ETOH
- 5ml 1M Tris pH 7.5
- 31.8ml water

0.5% Alizarin Red S stock: To make 50 mls add 0.25g Alizarin Red S (JT Baker cat. #A475-03) to 50 ml water.

0.02% Alizarin Stain/10% glycerol/0.5%KOH
To make 50 mls:
- 2ml 0.5% alizarin red S
- 5ml 100% glycerol
- 1.25ml 20% KOH
- 41.75ml water

10% glycerol/0.5%KOH
To make 50mls:
- 5ml 100% glycerol
- 1.25ml 20% KOH
- 43.75ml water

50% glycerol/0.5% KOH
To make 50 mls:
- 25ml 100% glycerol
- 1.25ml 20% KOH
- 23.75ml water

35% saturated sodium borate
To make 500mls: 8.3 g sodium borate decahydrate Q.S. to 500 ml with glass distilled water. Warm solution on hot plate with stirring until fully dissolved. This should take just a few minutes. Note: a saturated solution of sodium borate at 20°C is 23.6g/500ml water.

1% trypsin in 35% saturated sodium borate
Make fresh, using Difco trypsin 250 at 0.1g per 10mls 35% saturated sodium borate.

MESAB
Tricaine: 3-amino benzoic acid ethyl ester (SIGMA Cat # A-5040 or FINQUEL, Argent Laboratories #C-FINQ-UE-5G ).
Mix in fish safe container with a stir bar:
400 mg tricaine powder

800 mg Na2HPO4 (anhydrous)

100 ml glass distilled water

Adjust to ~pH 7 with a drop at a time of 1N NaOH or 1N HCl if needed but it's usually right if you weigh the sodium phosphate carefully and measure the water with a graduated cylinder.

8% PFA

8 g Pelleted PFA (Ted Pella, Inc.; cat# 18501)

90 ml dH2O

25 drops 1N NaOH

Heat at very low heat and stir just until sol'n clears. Q.S. 100 ml water.

Filter and store at 4C not more than 1 week.

Modified from:

_A two-color acid-free cartilage and bone stain for zebrafish larvae_  
MB Walker & CB Kimmel  