Kimmel Lab no acid bone & cartilage stain for larval fish

KIMMEL LAB STANDARD NO ACID DOUBLE BONE/CARTILAGE STAIN

PROTOCOL

for zebrafish larvae, 4-6 dpf:

1) MESAB embryos, fill tubes to the top with MESAB solution and lay on tubes on their sides for about 5 minutes; remove most of liquid.

2) Fix: Add 2% PFA/1X PBS. Nutate 1 hr, with tubes on their sides. Warning: Longer fixing may decrease bone staining.

3) Wash 1x10 mins with 100 mM Tris pH 7.5/10 mM MgCl2.

4) Alcian stain: Add 1 ml of 0.04% Alcian/10 mM MgCl2 stain pH 7.5. Nutate overnight. Warning: Embryos tend to get stuck in bottom of tube. Check for this more than once during staining. Wash with 80% ETOH/100 mM Tris pH 7.5/10 mM MgCl2 for 5 mins. Wash with 50% ETOH/100 mM Tris pH 7.5 for 5 mins. Wash with 25% ETOH/100 mM Tris pH 7.5 for 5 mins.

5) Bleach: Add 1 ml 3% H2O2/0.5% KOH. Let tubes sit with lids open, for 10 mins. Warning: Bleach can reduce bone staining.

6) Wash: 2x10 mins with 1 ml 25% glycerol/0.1% KOH. Rinse bleach out well. If bubbles are present, then bleach is not rinsed out enough.

7) Alizarin stain: Add 1 ml of 0.01% Alizarin stain, pH 7.5. Nutate 30 mins.

8) Destain: Add 1 ml 50% glycerol/0.1% KOH. Nutate 10 mins, then replace with fresh 50% glycerol/0.1% KOH. Warning: Some bone staining may fade if left on nutator overnight. If not viewing within a few hours, take embryos off nutator and store in rack, without agitation.

Solutions for larvae:

MESAB
Tricaine: 3-amino benzoic acid ethyl ester from Sigma (Cat # A-5040).
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.
For storage: Aliquot into 6 x 25 ml fish safe plastic bottles and store at 4C. Label with date made and use within a couple of weeks.

Dilution for babies: 10 drops in fish water (water in which fish are kept) in a 35 mm diameter disposable Petri dish. (~40-50 mls of liquid). Embryonic zebrafish can withstand being in MESAB for hours or until the anesthetic wears off. This will slow their growth rate, but does not seem to cause abnormal development of structures.

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 until sol’n clears.
Add 25 drops 1N HCl. pH should be 7.0-7.2.
Filter and store at 4C not more than 1 week.

2%PFA/1XPBS pH7.5: per ml:
250ul 8% PFA
100ul 10XPBS 650ul water

100 mM Tris pH 7.5/10 mM MgCl2:
To make 50 mls:
5ml 1M Tris pH 7.5
0.5ml 1 M MgCl2
44.5ml water
0.2% alcian blue/90%ETOH:
Alcian blue will not readily dissolve in 90% ETOH. To make 100 mls add 0.2g Alcian Blue 8GX (Anatech, Ltd. Cat.# 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.

0.04% Alcian/10mM MgCl2 Stain pH 7.5
To make 50 mls:
10ml 0.2% Alcian Blue in 90% ETOH
5ml 1M Tris pH 7.5 0.5ml 1M MgCl2
32.6ml 95% ETOH
1.9ml water

Ethanol rehydrations:
To make 50 mls:
80% ETOH/10mM MgCl2:
42.1ml 95% ETOH
5ml 1M Tris pH 7.5 0.5ml 1M MgCl2
2.4ml water
50% ETOH:
26.3ml 95% ETOH
5ml 1M Tris pH 7.5 5ml
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.

0.01% Alizarin Stain, pH 7.5
To make 50 mls:
1ml 0.5% Alizarin stock
12.5ml 100% glycerol
5ml 1M Tris pH 7.5
31.5ml water

3% H2O2/0.5% KOH: Mix just before using: 0.5ml 6%H2O2 & 0.5ml 1% KOH per microtube

25% glycerol/0.1% KOH
To make 50mls:
12.5ml 100% glycerol
250ul 20% KOH
37.25ml water

50% glycerol/0.1% KOH
To make 50 ml:
25ml 100% glycerol
250ul 20% KOH
24.75ml water