Alizarin Red Staining for Zebrafish Larvae

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Objective:

Detect calcification of developing vertebrae in zebrafish larvae as proof of bone development, using Calcium staining compound, Alizarin Red.

Sections:

Protocol:

I. Stock Solution Preparation:

1. Make 0.5% Alizarin Red in ddH_20 (0.5g/100mL ddH_20) stock solution.
2. Stir for 30-60 seconds, then place on shaker till powder dissolves completely, no filtration necessary.
3. Keep stock solution at RT no longer than 10 days.

*Stain older larvae as control, older larvae will ALWAYS stain red. When staining on older larvae if it doesn't work then reorder powder immediately! (Alizarin Red Sigma A5533-25G)

II. Zebrafish Larvae Preparation:

1. Fix the zebrafish larvae in 4% PFA for 3 - 4 hours at RT or overnight in cold room at 4°C.
2. Discard PFA in toxic waste container inside hood as it is a hazardous chemical.
3. Place on shaker and wash 3 times in PBST for 2-3 minutes per wash at RT. Discard first PBST wash waste in toxic waste container inside hood as it may contain trace amounts of PFA, a hazardous chemical.
4. Discard 2nd and 3rd PBST wash waste in non-hazardous liquid waste container.
5. Place on shaker and wash once for 10 minutes in 50% EtOH at RT.

6. Discard in non-hazardous liquid waste container.
7. Place on shaker and wash once for 10 minutes in PBST at RT.
8. Discard in non-hazardous liquid waste container.

III. Working Solution Preparation:

1. Make working concentration of Alizarin Red:

   2mL H_20

   2mL $MgCl_2$ (1M)

   7mL 100% EtOH

   9mL 70% EtOH

   500µL 0.5% Alizarin Red Stock Solution

IV. Staining

1. Stain using working concentration of Alizarin Red for 1 – 2 days at RT in the dark (cover with Aluminum foil).
2. Discard in separate non-hazardous waste container designated for Alizarin Red.
3. Place on shaker and wash 2 times in 0.1% Tween in ddH_20 for 2 – 3 minutes per wash.
4. Discard in non-hazardous liquid waste container.
5. Place on shaker and bleach for 10 – 15 minutes (do NOT bleach for more than 20 minutes) using $3\%H_2O_2/2\%KOH$ in a 1:1 ratio.
6. Discard in non-hazardous liquid waste container.
7. Wash once with 0.1% Tween in ddH_20 for 2 – 3 minutes.
8. Discard in non-hazardous liquid waste container.
9. Image samples or keep in 4°C until ready for imaging.
10. Store zebrafish larvae in the dark as light will bleach the staining!
11. When imaging place samples in 3% methylcellulose and laterally orient larvae.
12. Take 5 images per sample to account for human error during imaging.