

C-204 MODIFIED ALIZARIN RED S FOR FETAL SPECIMENS

Fixation: Two or more weeks in 95% alcohol or Formalin after evisceration.
(evisceration omitted for small embryos).

PROCEDURE:

1. Rinse in water and place in **Potassium Hydroxide, 1%** (C-204-2) for a few hours to a week or more (Formalin fixed specimens require approximately 1 month in **Potassium Hydroxide, 10%** (C-204-2A) until bones are clearly viable through the soft tissue.
Note: If the tissue becomes too soft, harden for 12-24 hours in solution containing equal parts of 95% alcohol, water and glycerin and then return to the **Potassium Hydroxide 1%**, (C-204-2)
The **Potassium Hydroxide, 1%** (C-204-2) may be diluted to 0.5% with distilled water during the last few days of clearing.
2. Wash in running water for 12 hours.
4. Stain in Working Alizarin Red S Solution* for 30-60 minutes. Wash in running water for 30 minutes.
*Prepare Working Stain by adding 6-10 drops of **Potassium Hydroxide, 1%** (C-204-2) to 100 ml of **Alizarin Red S, 0.01%** (C-204-1). Prepare fresh.
5. Decolorize in **Glycerol-Potassium Hydroxide Solution** (C-204-3) for 1-2 weeks.
6. Mount on a glass frame and dehydrate by passing slowly through the following alcohol-glycerin-water mixtures, in the order given: 1:2:7, 2:2:6, 3:3:4, 4:4:2 and finally 5:5 without water.
7. Seal in the final glycerin-alcohol mixture.

Stain Results:

Bones	Red
Soft Tissue	Transparent and unstained

References:

- Dawson, A.B., Stain Techn., 1:123-4, 1926
Richmond, G.W. and Bennett, L., Stain Techn., 13:77-79, 1938.
Clark, G., (ed.), Staining Procedures, 3rd edition, Williams & Wilkins, Baltimore, p.137, c. 1973.

