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## **C-205 ALIZARIN RED S AND TOLUIDINE BLUE O**

**FIXATION:** At least 1 week in **10% Formalin** (F-111) after evisceration and washing.  
(Evisceration is not necessary for museum specimen.)

**STAINING:**

1. Wash the specimen in 250ml of **Ammonium Alcohol** (C-205-3) for 24 hours.
2. Stain in **Toluidine Blue Solution 0.25%** (C-205-2) for 1 week.
3. Harden and destain for 72 hours in 4 changes of 95% alcohol.
4. Macerate in several changes of **Potassium Hydroxide, 2%** (C-205-5) for 5-7 days, depending on the size of the animal. This step is facilitated by exposure to sunlight or ultraviolet rays.
5. Transfer to Working Alizarin Red S solution for approximately 24 hours.

\*Prepare the Working Stain by mixing equal parts of **Alizarin Red S, 0.002%** (C-205-1) and **Potassium Hydroxide, 4%** (C-205-1A). Prepare fresh.

If specimen has been insufficiently macerated the soft tissues will appear slightly stained. They may be quickly destained in **Sulfuric Acid-Alcohol** (C-205-6).

6. Dehydrate through 3 changes of Cellosolve, 6 hours each. Reduce the time for small embryos.

OPTIONAL: One may substitute the alcohol series, 50%, 80% and 90% followed by 3 changes of benzene.

7. Clear by transferring to solutions for 25%, 50% and the 75% methyl salicylate in Cellosolve for 24 hours each. Then store in methyl salicylate.

*Modification using glycerin for clearing:* Omit step 6 and transfer directly from Working Alizarin Red S solution into a series of 50%, 70% & 80% glycerin Solutions for 24 hours each. Store in glycerin.

**Stain Results:**

Soft tissues	Transparent
Osseous Tissues	Deep Red
Cartilage	Dark Blue

NOTE: The intensity of the stains serves to indicate the relative amounts of ossification and chondrogenesis which may be taken place. The bone cartilage may be stained separately by omitting step 2 for bone or step 5 for cartilage.

**References:**

Dawson, A.B., Stain Techn., 1:123-4, 1926.

Williams, T.W., Stain Techn., 16:23-5, 1941

Clark, G., (ed.), Staining Procedures, 3<sup>rd</sup> ed., Williams & Wilkins Baltimore, p. 139, c. 1973.