

---

---

**ALIZARIN RED S - CALCIUM**

---

---

**PURPOSE:** To identify calcium in tissue sections.

**PRINCIPLE:** Calcium forms an alizarin red S-calcium complex in a chelation process. The reaction is birefringent.

**CONTROL:** A known calcium containing tissue section.

**FIXATIVE:** 10% formalin, or alcoholic formalin.

**TECHNIQUE:** Cut paraffin sections 4 $\mu$ .

**EQUIPMENT:** Rinse all glassware in DI water: coplin jars, pH meter, bibulous paper, microscope, polarizing lenses.

**REAGENTS:**

**Alizarin Red S Solution:**

Alizarin red S                      2.0 gm  
Distilled water                      100.0 ml

Mix the solution, adjust the pH to 4.1-4.3 using 0.5% ammonium hydroxide. The pH is critical, make fresh.

**CAUTION:** Avoid contact and inhalation.

**Acetone**

**CAUTION:** Flammable.

**Acetone-Xylene:**

Acetone                                      25.0 ml  
Xylene                                        25.0 ml

Make fresh.

**CAUTION:** Flammable.

**SAFETY:** Wear gloves, goggles and lab coat. Avoid contact and inhalation.

Sodium hydroxide; severe irritant to skin and eyes. Corrosive, can cause burns.

Acetone; moderately toxic on ingestion. May cause dizziness, headaches and irritation to respiratory system on inhalation. Defatting and dermatitis on skin contact. Flammable

**PROCEDURE:**

1. Deparaffinize and hydrate to 70% alcohol.
2. Rinse rapidly in distilled water.
3. Alizarin red S solution, 30 seconds to 5 minutes, checking microscopically for an orange-red color.
4. Shake off excess dye, blot sections.
5. Acetone, 20 dips.
6. Acetone-xylene, 20 dips.
7. Clear in xylene, mount in PERMOUNT.

**RESULTS:**

Calcium deposits: orange-red

**REFERENCES:**

- Carson F, Histotechnology: A Self-Instructional Text, 1990, pp 226-227, ASCP, III
- Fredenburgh, JL, Grizzle, WE, Safety and Compliance In the Histology Laboratory: Biohazards to Toxic Chemicals, NSH 1993, Workshop

Prepared: \_\_\_\_\_ By: \_\_\_\_\_  
Approved: \_\_\_\_\_ By: \_\_\_\_\_

## PROCEDURE CARD

### **ALIZARIN RED S - CALCIUM**

---

**CONTROL:** A known calcium containing tissue section.

**TECHNIQUE:** Cut paraffin sections 4 $\mu$ .

#### **PROCEDURE:**

1. Deparaffinize and hydrate to 70% alcohol.
2. Rinse rapidly in distilled water.
3. Alizarin red S solution, 30 seconds to 5 minutes, checking microscopically for an orange-red color.
4. Shake off excess dye, blot sections.
5. Acetone, 20 dips.
6. Acetone-xylene, 20 dips.
7. Clear in xylene, mount in PERMOUNT.

#### **RESULTS:**

Calcium deposits: orange-red

#### **Alizarin Red S Solution:**

Alizarin red S	2.0 gm
Distilled water	100.0 ml

Mix the solution, adjust the pH to 4.1-4.3 using 0.5% ammonium hydroxide. The pH is critical, make fresh.

**CAUTION:** Avoid contact and inhalation.

#### **Acetone**

**CAUTION: Flammable.**

#### **Acetone-Xylene:**

Acetone	25.0 ml
Xylene	25.0 ml

Make fresh.

**CAUTION: Flammable.**