

Calcium Staining Solution (Alizarin Red S Method)

P1508560

Storage: Room Temperature.

Introduction:

Calcium is abundant in the human body, forming the skeleton that provides structural support and playing important roles in secretion, transport, muscle contraction, and nerve conduction. Calcium exists in two forms within the body: one is ionic calcium, present in the bloodstream (referred to as blood calcium); the other is bound calcium, which combines with proteins, carbonates, or phosphates and deposits in tissues. Aside from bones and teeth, calcium normally permeates all tissues and cells and generally does not appear as a solid within tissues. However, under certain conditions, calcium may precipitate as a solid and deposit in tissues, leading to pathological calcification. The deposited calcium salts are primarily calcium phosphate, followed by calcium carbonate. Calcium salts are usually monorefringent, but calcium oxalate is birefringent. When stained with HE, calcium typically appears purplish-blue. Many dyes can form chelates with calcium, including Alizarin Red S, purpurin, nuclear fast red, etc. Generally, these dyes are more effective in identifying moderate to large amounts of calcium than in staining trace calcium deposits lightly, but Alizarin Red S often yields more reliable results for small amounts of deposits.

Common methods for calcium salt staining include the silver nitrate method and the Alizarin Red S method. This product uses a combined staining method with Alizarin Red S and Fast Green FCF. Alizarin Red S is an anthraquinone derivative and the sodium salt of alizarinsulfonic acid, which can chelate with calcium salts in calcium carbonate or calcium phosphate to form an orange-red complex; the combination of calcium salts and Alizarin Red produces an orange-red precipitate, suitable for staining tissues with small amounts of calcium salts. This reagent is intended for research use only and is not suitable for clinical diagnosis or other purposes.

Kit Contents and Storage Conditions:

P1508560	Component	2×50mL	Storage
P1508560A	Alizarin Red S Staining Solution	50 mL	RT
P1508560B	Fast Green FCF Staining Solution	50 mL	RT

Reagents, consumables and Equipments not provided:

- Fixatives: 10% neutral formalin, ethanol-formalin, ethanol, etc.
- Graded ethanol series, distilled water, xylene or dewaxing clearing solution, neutral balsam, acetic acid aqueous solution
- Microscope.

Instructions for Use (For Reference Only):

1. Routine Staining:

- Fix tissue in 10% neutral formalin or ethanol, followed by routine dehydration and embedding.
- Section to 95% ethanol; place slides upright and allow to dry completely.
- Immerse sections in a staining jar containing Alizarin Red S Staining Solution for 1–5 minutes (see Note 1); rinse quickly with distilled water.
- Counterstain with Fast Green FCF Staining Solution for 1 minute; rinse with distilled water three times.
- Routine dehydration, clear with xylene or dewaxing clearing solution, and mount with neutral balsam.

2. Insoluble Calcium Staining:

- Fix tissue in 10% neutral formalin fixative and dehydrate routinely, ensuring thorough dehydration in 95% ethanol
- Embed in plastic and leave overnight at room temperature; polymerize in a 37°C oven for 2–4 days; cool in a –20°C freezer for 15–20 minutes.
- Section to a thickness of 3–5 µm; for undecalcified bone sections, remove plastic and bring to water; wash with distilled water.
- Immerse sections in Alizarin Red S Staining Solution for 5–10 minutes (see Note 2); wash with distilled water
- Counterstain with Fast Green FCF Staining Solution for 30–60 seconds.
- Rinse quickly with 0.2–1% acetic acid aqueous solution; wash briefly under running water.
- Blot dry with filter paper; decolorize with 95% ethanol.
- Routine dehydration, clear with xylene or dewaxing clearing solution, and mount with neutral balsam.

Staining Results:

Calcium deposits	Orange-red
Background	Green

Notes:

1. The staining time with Alizarin Red S should be determined based on the calcium salt content. Observe under a microscope and remove the slide for washing when calcium salts appear a deep orange-red. Over-staining may cause diffusion; generally, 1–2 minutes is sufficient.
2. For insoluble calcium staining, the staining time with Alizarin Red S should be determined based on the calcium salt content. Observe under a microscope until the staining reaction is strong without diffusion.
3. After staining with Alizarin Red S, calcium deposits are birefringent.
4. This method is particularly useful for identifying and detecting small amounts of calcium, such as abnormal calcification in the kidneys (hypercalciuria), which appears orange-red.

5. Use the reagents as soon as possible after opening to avoid affecting subsequent experimental results.

aladdin®