
Alizarin Red S Staining Solution (2%, pH4.2)

A774531

Storage: Room Temperature. Store in the dark.

Introduction:

Calcium is abundant in the human body, forming the skeleton that serves as the supporting framework. It also plays important roles in secretion, transport, muscle contraction, and nerve conduction. Calcium exists in the body in two forms: one is ionic calcium, present in the bloodstream and 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, and others.

Alizarin Red S staining is a commonly used staining method widely employed in the study of osteoblast differentiation, bone cells, or tissue pathophysiology. Alizarin Red S (abbreviated as ARS), also known as sodium alizarinsulfonate or alizarin carmine, has a CAS number of 130-22-3, molecular formula $C_{14}H_7NaO_7S$, and molecular weight 342.25. Alizarin Red S is an anthraquinone derivative that can chelate with various metal ions to form stable orange-red or deep red complexes. It serves as an excellent metal indicator, a chromogenic agent for spectrophotometric determination of metal ions, an acid-base indicator, and is used in polarographic determination of metal ions. In histology and histopathology, because Alizarin Red S can chelate with calcium salts in calcium carbonate or calcium phosphate to form an orange-red complex, it is often used to stain or localize calcium deposits in tissues.

Instructions for Use:

1. Fix tissue in 10% neutral formalin or ethanol, followed by routine dehydration and embedding.
2. Section to 95% ethanol.
3. Place slides upright and allow to dry completely.
4. Immerse sections in a staining jar containing Alizarin Red S staining solution for 5–20 minutes (see Note 1).
5. Rinse quickly with distilled water.
6. (Optional) Counterstain with a counterstaining solution, then rinse with distilled water three times.
7. Routine dehydration and clearing, mount with neutral balsam.

Staining Results:

Calcium deposits	Orange-red
Background	Varies depending on the counterstain used

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; typically, 1–2 minutes is sufficient.
2. After staining with Alizarin Red S, calcium deposits are birefringent.
3. When Fast Green FCF is used as the counterstain, the background appears green. When Mayer's hematoxylin is used as the counterstain, the nuclei appear blue.
4. The Alizarin Red S method is particularly useful for identifying and detecting small amounts of calcium, such as examining abnormal calcification in the kidneys (hypercalciuria).
5. For your safety and health, please wear a lab coat and disposable gloves during operation.