

Calcium Salt Staining Solution (Modified Scharlach Red S Method)

Cat #: A-CSH335

Size: 3×50mL

Storage: 2~8°C, avoid light (12 months)

Product Composition

Component	3×50mL	Store at
Reagent (A): Alizarin Red S staining solution	50ml	10~30°C, and were protected from light
Reagent (B): McGee-Russell differentiation solution	50ml	10~30°C, and were protected from light
Reagent (C): Mayer hematoxylin staining solution	50ml	2~8°C, and were protected from light

Product Description

Calcium exists in large quantities in the human body, forming the skeletal framework that serves as the support structure. It also plays a crucial role in secretion, transportation, muscle contraction, and nerve conduction. Calcium exists in two forms within the body: ionized calcium, which circulates in the bloodstream (referred to as blood calcium), and bound calcium, which combines with proteins, carbonates, or phosphates and deposits in tissues. Apart from bones and teeth, calcium normally permeates all tissues and cells in a non-solid state. However, in certain circumstances, calcium can precipitate and deposit as a solid in tissues, leading to pathological calcium salt deposition. The primary calcium salts deposited are calcium phosphate, followed by calcium carbonate. Calcium salts are typically uniaxial, but calcium oxalate is birefringent. When using HE staining, calcium generally appears purple-blue. Many dyes can form chelates with calcium, including Alizarin Red S, Eriochrome Red B, and Nuclear Fast Red.

Alizarin Red S is a derivative of anthraquinone and the sodium salt of alizarin sulfonic acid. It can chelate with calcium salts in calcium carbonate or calcium phosphate to form an orange-red complex. Generally, these dyes are more effective in identifying large amounts of calcium compared to the slight staining of trace calcium deposits. However,

Alizarin Red S staining often provides more reliable results for staining small amounts of deposits.

The staining of calcium salts is commonly performed using the silver nitrate method and the Alizarin Red S method. This staining solution uses a modified McGee-Russell method, employing Alizarin Red S and Mayer's hematoxylin stain, especially suitable for staining tissues with small amounts of calcium salts.

Usage

(I) Routine Staining:

1. Fix the tissue in 10% neutral formalin fixative and perform routine dehydration and embedding.
2. Cut sections with a thickness of 5µm and perform routine dewaxing until water.
3. Drop the sections into Alizarin Red S staining solution and stain for 1-5 minutes (see Note 1), then rinse briefly with water.
4. Differentiate the sections rapidly in McGee-Russell differentiation solution for a few seconds.
5. Lightly stain the cell nuclei with Mayer's hematoxylin staining solution for 1-2 minutes, followed by a 10-minute water rinse.
6. Perform routine dehydration, clearing, and mount with a neutral mounting medium.

(Note 1: The staining time with Alizarin Red S staining solution should be determined based on the desired results.

When the calcium salts exhibit a deep orange-red color, remove the sections and rinse with water. Generally, 1-5 minutes is sufficient.)

(II) Insoluble Calcium Staining:

1. Fix the tissue in 10% neutral formalin fixative, perform routine dehydration, and ensure thorough dehydration in 95% ethanol.
2. Embed the tissue in plastic, leave it at room temperature overnight, polymerize in a 37°C oven for 2-4 days, and cool in a -20°C freezer for 15-20 minutes.
3. Cut sections with a thickness of 3-5µm. For decalcified bone sections, dewax them until water and rinse with distilled water.
4. Immerse the sections in Alizarin Red S staining solution for 1-10 minutes (see Note 2), then rinse with distilled water.

5. (Optional) Rapidly differentiate the sections in McGee-Russell differentiation solution for a few seconds.
6. (Optional) Lightly stain the cell nuclei with Mayer's hematoxylin staining solution for 1-2 minutes, followed by a 10-minute water rinse.
7. Perform routine dehydration, clearing, and mount with a neutral mounting medium.

(Note 2: The staining time with Alizarin Red S staining solution should be determined based on the desired results. When the calcium salts exhibit the desired staining intensity, remove the sections and rinse with distilled water. Generally, 1-10 minutes is sufficient.)

Staining Results

Calcium sediment	orange red
nucleus	blue

Notes

1. The staining time with Alizarin Red S should be determined based on the content of calcium salts. When observing under a microscope, if the calcium salts appear as a deep orange-red color, remove the sections and rinse with water. Prolonged staining time may lead to diffusion. Generally, 1-2 minutes is sufficient.
2. In the staining of insoluble calcium, the staining time with Alizarin Red S should be determined based on the content of calcium salts. This method is commonly used in pathological diagnosis and scientific research of pathological calcification and ossification, such as fracture repair, ossifying myositis, and fixation of old scar tissue.
3. Differentiation with McGee-Russell solution and staining with Mayer's hematoxylin are not mandatory steps.
4. After staining with Alizarin Red S, calcium deposits exhibit birefringence.
5. The Alizarin Red S method is particularly useful for distinguishing and detecting small amounts of calcium, such as examining abnormal calcification (excessive urinary calcium) in the kidneys.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.