

Calcium Stain Kit (Modified Alizarin Red S Method)

Introduction

Calcium is a critical structural component of bone and is essential for secretion, transport, muscle contraction, and nerve conduction. It circulates as ionic calcium (blood calcium) or is bound to proteins, carbonates, or phosphates. Under normal conditions, calcium is diffusely distributed; however, pathological calcification can occur when calcium precipitates as solid deposits, primarily composed of calcium phosphate or calcium carbonate.

Several dyes form stable chelates with calcium, including Alizarin Red S, Purpurin, and Nuclear Fast Red. Alizarin Red S (an anthraquinone derivative) binds calcium carbonate and phosphate to form a distinct orange-red complex. Compared to other dyes, Alizarin Red S provides superior sensitivity for detecting small calcium deposits.

This kit is based on the modified McGee-Russell technique. The kit consists of Alizarin Red S and Mayer's Hematoxylin. Calcium salts form an orange-red complex upon reaction with Alizarin Red S, while Mayer's Hematoxylin counterstains nuclei blue. This formulation is particularly suitable for visualizing tissues with minimal calcium deposits.

Components and Storage

| Components | Size | Storage |
|---|-------|----------------------|
| Reagent (A): Alizarin red S staining solution | 50 mL | 4°C away from light |
| Reagent (B): McGee-Russell differentiation solution | 50 mL | 4°C away from light |
| Reagent (C): Mayer Hematoxylin staining solution | 50 mL | 4°C away from light |
| Shipping: Blue ice | | Shelf life: 6 months |

Protocol

1. Materials Required but Not Included

- 1) 10% Neutral Buffered Formalin
- 2) Graded series of ethanol
- 3) Distilled water

2. Fix tissue in 10% Neutral Buffered Formalin and process routinely for dehydration and embedding.
3. Cut sections at 5 μ m and deparaffinize to water.
4. Stain sections with Alizarin red S staining solution for 1-5 min. Rinse quickly with distilled water.

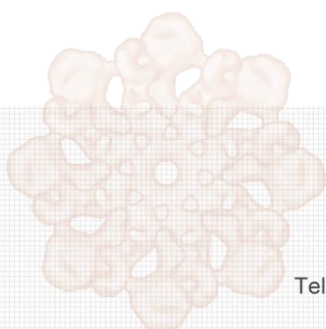
Note: The staining time for Alizarin Red S depends on the calcium content and should be monitored under a microscope. Remove the slide and rinse with water when the calcium deposits turn a deep orange-red. Prolonged staining may cause diffusion; generally, 1-2 min is sufficient.

5. Differentiate quickly in McGee-Russel1 differentiation solution for a few seconds.
6. Counterstain nuclei lightly with Mayer Hematoxylin staining solution for 1-2 min. Rinse under running tap water for 10 min.
7. Dehydrate, clear, and mount with neutral balsam.
8. Staining results

| | |
|------------------|------------|
| Calcium Deposits | Orange-Red |
| Nuclei | Blue |

Note

1. Calcium deposits exhibit birefringence after staining with Alizarin Red S.
2. The Alizarin Red S method is particularly useful for identifying and detecting minimal amounts of calcium, such as abnormal calcification in the kidneys (due to hypercalciuria).
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.



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