Calcium Stain Kit (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 utilizes a combined Alizarin Red S/Fast Green staining protocol. Alizarin Red S yields a sharp orange-red precipitate at calcification sites, while Fast Green provides a contrasting green background, making it particularly suitable for visualizing minimal calcium deposits.

Components and Storage

Components \ Size	50 mL	Storage
Reagent (A): Alizarin Red S staining solution	50 mL	4°C away from light
Reagent (B): Fast Green FCF dyeing 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. Deparaffinize sections to 95% ethanol. Place slides vertically and allow to air dry.

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. Immerse in Fast Green FCF dyeing solution for 1 min. Rinse with distilled water 3 times, 1 min each.

6. Dehydrate, clear, and mount with neutral balsam.

7. Staining results

Calcium Deposits	Orange-Red
Background	Green

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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