Von Kossa Calcium Staining Kit

Catalog No.: RA20130

Basic Information

Product name	Von Kossa Calcium Staining Kit
Sizes	2x 50 mL
Storage	2-8 ℃, keep away from light
Shipping	Shipped with ice pack
Validity	12 months

Product Introduction

Calcium is abundant in the human body, forming the skeleton as a structural support. It also plays vital roles in secretion, transport, muscle contraction, and nerve conduction. Calcium exists in two forms: Ionic calcium – found in blood circulation (i.e., blood calcium); Bound calcium – combined with proteins, carbonates, or phosphates and deposited in tissues. Apart from bones and teeth, calcium is normally present in all tissues and cells, usually not in solid form. However, under certain pathological conditions, calcium may precipitate and deposit in tissues as solid particles, a process known as pathological calcification. The deposited calcium salts are mainly calcium phosphate, followed by calcium carbonate. Calcium salts are usually mono-refringent, except for calcium oxalate, which is bi-refringent. In H&E staining, calcium generally appears purple-blue. Many dyes can form chelates with calcium, including Alizarin Red S, Purpurin, and Nuclear Fast Red. Alizarin Red S, an anthraquinone derivative and sodium salt of alizarin sulfonate, can chelate with calcium in calcium carbonate or phosphate to form an orange-red complex.

Common calcium staining methods include the silver nitrate method and Alizarin Red S method. Von Kossa staining (silver method) is a metal substitution technique. When Von Kossa silver solution reacts with sections containing insoluble calcium salts, calcium is replaced by silver. Upon light exposure, the silver salt is reduced to black metallic silver, making this method suitable for high-throughput calcium staining in tissue sections.

Product Components

Components	2x 50mL
Reagent (A): Von Kossa Silver Solution	50 mL

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Reagent (B): Hypo Solution	50 mL
Reagent (C): Von Kossa Control Solution	10 mL

Materials Required (Not Supplied)

1. 10% neutral buffered formalin (NBF), graded ethanol, distilled water, eco-friendly dewaxing agent or xylene, neutral balsam.
2. H&E or Van Gieson staining solution, lithium carbonate aqueous solution.

Perimental procedure

(I) Routine Staining

1. Fix tissue in 10% neutral buffered formalin, then dehydrate and embed routinely.
2. Cut sections at 3–5 μm. Dewax with xylene or eco-friendly dewaxing agent, hydrate to water, and rinse with distilled water.
3. Immerse sections in Von Kossa Silver Solution, expose to strong light for 15–60 min (see Note 2), then rinse with distilled water for 1 min.
4. Treat with Hypo Solution for 2 min, then counterstain nuclei with H&E or Van Gieson for 1 min.
5. Dehydrate routinely, clear with xylene or eco-friendly clearing agent, and mount with neutral balsam.

(II) Insoluble Calcium Staining

1. Fix tissue in 10% neutral buffered formalin, dehydrate routinely. Ensure sufficient dehydration in 95% ethanol.
2. Embed in plastic, leave at room temperature overnight, then polymerize at 37 °C for 2–4 days. Cool at –20 °C for 15–20 min.
3. Cut undecalcified bone sections at 3–5 μm. Dewax (or deplasticize) to water, rinse with distilled water.
4. Immerse in Von Kossa Silver Solution, expose to strong light for 10–60 min (see Note 2), rinse with distilled water.
5. Treat with Hypo Solution for 2 min, counterstain nuclei with H&E, Neutral Red, or Van Gieson for 1 min.
6. Dehydrate routinely, clear with xylene or eco-friendly clearing agent, and mount with neutral balsam.

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Staining Results

Component	Color
Calcium salts, urates	Dark brown to black
Nuclei	Varies depending on counterstain

Negative Control

If necessary, perform a negative control. Take an adjacent section, dewax (or deplasticize) to water, treat with Von Kossa Control Solution for 10–20 min, rinse with distilled water, then proceed with the same staining protocol. The result should be negative.

Notes

1. Neutral buffered formalin is the preferred fixative for calcium-containing tissues. Avoid acidic fixatives such as Bouin' s solution or calcium-formalin. If using routine 10% formalin, limit fixation to 4–6 h before dehydration to prevent acidification-induced calcium dissolution.
2. Reaction time depends on light intensity and exposure duration: 15 min under strong sunlight is usually sufficient; 10 min under UV light is adequate; Under normal indoor light, extend exposure time accordingly.
3. If calcium staining is too intense, dilute the Von Kossa Silver Solution with distilled water, or reduce light exposure time/intensity. If staining is too weak, increase the volume of silver solution and extend light exposure time and intensity.
4. This method can differentiate calcium salts from urates: Calcium salts are insoluble in lithium carbonate solution; Urates are soluble. Therefore, treat sections with lithium carbonate before Von Kossa staining and light exposure. Negative staining indicates urate deposition.
5. Use reagents promptly after opening to maintain optimal performance.

This product is for research use only!