
Calcium Staining Solution (Modified Alizarin Red S Method)

Cat. No.: C774863 | Pack size: 3×50 mL | Storage: Store at 2-8°C, Protected from light, Room temperature

Overview

Calcium is abundant in the human body, forming bones that serve as the body's supporting framework, and also plays crucial 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 (so-called blood calcium); the other is bound calcium, which combines with proteins, carbonate, or phosphate and deposits in tissues. Apart from bones and teeth, calcium normally permeates all tissues and cells and generally does not appear in a solid state within tissues. However, under certain pathological conditions, calcium can precipitate into solids and deposit within tissues, known as 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 H&E, calcium generally appears purplish-blue. Many dyes can chelate with calcium, including Alizarin Red S, purpurin, nuclear fast red, etc. Alizarin Red S is an anthraquinone derivative, the sodium salt of alizarinsulfonic acid, which chelates with calcium salts in calcium carbonate or calcium phosphate to form an orange-red complex. Generally, these dyes are more effective in identifying moderate to large amounts of calcium than faintly stained micro-deposits, but Alizarin Red S often yields more reliable results for small amounts of deposits.

Common methods for calcium salt staining include the silver nitrate method and the Alizarin Red S method. This staining solution uses the modified McGee-Russell method with Alizarin Red S and Mayer's hematoxylin staining, particularly suitable for staining tissues with small amounts of calcium salts.

Instructions for Use

I. Conventional Staining

Fix tissue in 10% neutral buffered formalin, followed by routine dehydration and embedding.

Cut sections at 5 µm thickness, and routinely deparaffinize to water.

Cover the section with Alizarin Red S Staining Solution and stain for 1~5 minutes (see Note 1). Rinse briefly with water.

Differentiate rapidly with McGee-Russell Differentiation Solution for a few seconds.

Counterstain nuclei lightly with Mayer's Hematoxylin Staining Solution for 1~2 minutes. Rinse under running tap water for 10 minutes.

Dehydrate and clear routinely, then mount with neutral balsam.

II. Insoluble Calcium Staining (e.g., for Undecalcified Bone)

Fix tissue in 10% neutral buffered formalin and dehydrate routinely. Ensure thorough dehydration in 95% ethanol.

Embed in plastic resin and polymerize overnight at room temperature, then in a 37°C oven for 2~4 days. Cool in a -20°C freezer for 15~20 minutes.

Cut sections at 3~5 µm thickness. Depolymerize undecalcified bone sections to water, then rinse with distilled water.

Immerse sections in Alizarin Red S Staining Solution for 1~10 minutes (see Note 2). Rinse with distilled water.

(Optional) Differentiate rapidly with McGee-Russell Differentiation Solution for a few seconds.

(Optional) Counterstain nuclei lightly with Mayer's Hematoxylin Staining Solution for 1~2 minutes.

Rinse under running tap water for 10 minutes.

Dehydrate and clear routinely, then mount with neutral balsam.

Staining Results

Target	Result
Calcium Deposits	Orange-red
Cell Nuclei	Blue

Precautions

The staining time with Alizarin Red S should be determined based on the calcium salt content. Observe under a microscope and stop when calcium salts appear a deep orange-red, then rinse with water. Over-staining may cause diffusion; generally, 1~2 minutes is sufficient.

For insoluble calcium staining, the Alizarin Red S staining time should also be determined by calcium salt content. This method is commonly used in pathological diagnosis and research of pathological calcification and ossification, such as fracture repair, myositis ossificans, and calcification in old scar tissue.

Steps involving McGee-Russell differentiation and Mayer's hematoxylin counterstaining are not mandatory.

The Alizarin Red S method is particularly useful for identifying and detecting small amounts of calcium, such as checking for abnormal calcification in the kidney (nephrocalcinosis).

Key Features

- Supplied as BioReagent, for microscopy, Biological Stain grade material for research workflows.

- Designed for use in Cell Staining applications.
- Available pack size: 3×50 mL.
- Storage condition: Store at 2-8°C, Protected from light, Room temperature.
- Related name(s): Calcium Stain Kit (Modified Alizarin Red S Method).
- For research use only; not intended for clinical diagnosis, treatment, food, or household applications.

Contents & Storage

Cat. No.	Component / Product	Size	Storage
C774863A	Alizarin Red S Staining Solution	50 mL	RT. Store in the dark.
C774863B	McGee-Russell Differentiation Solution	50 mL	RT. Store in the dark.
C774863C	Mayer's Hematoxylin Staining Solution	50 mL	2-8°C. Store in the dark.

Shelf life: Each component has a shelf life of 1 year under corresponding storage conditions.

Shipping: Wet ice This product requires cold chain shipping. Ground and other economy services are not available.

Product Information

Item	Description
Cat. No.	C774863
Product Name	Calcium Staining Solution (Modified Alizarin Red S Method)
Specifications & Purity	BioReagent, for microscopy, Biological Stain
Synonyms	Calcium Stain Kit (Modified Alizarin Red S Method)
Pack Size	3×50 mL
Storage	Store at 2-8°C, Protected from light, Room temperature
Stability & Storage	Each component has a shelf life of 1 year under corresponding storage conditions.
Shipped In	Wet ice. This product requires cold chain shipping. Ground and other economy services are not available.
Recommended Application	Cell Staining

Chemical and Physical Properties

Property	Value
Sensitivity	Light-sensitive

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Personal protective equipment	Lab coat, disposable gloves, eye protection	Safe handling and contamination control
Microscope or imaging system	Configured for the intended staining or observation workflow	Signal observation and documentation
Pipettes and tips	Clean disposable tips	Reagent and sample handling
PBS, distilled water or normal saline	As required by the source procedure	Washing, dilution or sample preparation

Safety & Precautions

- The staining time with Alizarin Red S should be determined based on the calcium salt content. Observe under a microscope and stop when calcium salts appear a deep orange-red, then rinse with water. Over-staining may cause diffusion; generally, 1~2 minutes is sufficient.
- For insoluble calcium staining, the Alizarin Red S staining time should also be determined by calcium salt content. This method is commonly used in pathological diagnosis and research of pathological calcification and ossification, such as fracture repair, myositis ossificans, and calcification in old scar tissue.
- Steps involving McGee-Russell differentiation and Mayer's hematoxylin counterstaining are not mandatory.
- The Alizarin Red S method is particularly useful for identifying and detecting small amounts of calcium, such as checking for abnormal calcification in the kidney (nephrocalcinosis).
- Wear a lab coat, disposable gloves and eye protection during handling.
- Use only under an approved research protocol and in accordance with applicable institutional guidelines.

Quality Control

QC Item	Method	Acceptance Criteria
Visual inspection on receipt	Confirm that the container is intact and the label, lot number, pack size and expiry information are legible.	No leakage, visible damage or labeling inconsistency.
Product identity and specification	Check the product label and lot-specific COA against the product information in this manual.	Product name, Cat. No., grade/purity and storage condition match the ordered product.
Appearance / usability	Inspect before use according to the source precautions and applicable laboratory SOPs.	No abnormal precipitation, discoloration, contamination or degradation that would interfere with the intended assay.
Staining performance	Evaluate positive/negative or expected sample response under the recommended observation conditions.	Expected staining pattern is observed with acceptable background.

Troubleshooting

Issue	Possible Causes	Corrective Action
Weak or uneven staining / signal	Insufficient staining time, incomplete sample coverage, quenching, expired or improperly stored reagent	Ensure complete sample coverage, follow the source procedure, protect fluorescent dyes from light, and use fresh or properly stored reagent.
High background	Excess staining solution, inadequate washing, overly long staining time	Optimize staining duration, wash as recommended, and avoid over-staining.
Signal fades quickly	Photobleaching or repeated freeze-thaw cycles	Protect from light, minimize exposure during observation, and avoid repeated freeze-thaw cycles where applicable.

Recommended Applications

Cell Staining.

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, please contact Aladdin Scientific through the offices below.

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Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, food, cosmetic, or household applications unless specifically authorized by applicable documentation.
- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared or FDA-approved diagnostic product.
- Where any product or component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), users must follow the current SDS, product label and local regulations.
- Performance depends on sample type, sample condition, storage, handling, and operator technique. Users are responsible for validating suitability for their intended research application.
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