

SPECIAL STAINS
Alizarin Red S for Calcium
Cat. No. SS052-50K

Doc. No. 932-SS052-50K Rev. No. A
Release Date: 19-Jun-14

Intended Use

This product is intended for in vitro diagnostic use for demonstrating deposits of calcium in tissues.

Summary and Explanation

Alizarin Red S is an anthraquinone derivative and is used to identify calcium in tissue sections. The reaction is not strictly specific for calcium. Magnesium, manganese, barium, strontium, and iron may interfere, but these elements usually do not occur in sufficient concentration to interfere with the staining.

Principles of the Procedure

Calcium forms an alizarin red S-calcium complex in a chelating process. The reaction is birefringent.

Reagents Provided

Alizarin Red Solution (HK984-14K)	1 x 13 ml, ready to use
Acetone-Xylene Mix (HK985-14K)	2x13ml, ready to use

Reagents Required but Not Supplied

Acetone
Xylene
Mounting Medium
Positive Control Tissue (FS052)

Storage and Handling

Store all reagents at 2-8°C. Do not use after expiration dates as indicated on the reagent labels.

Specimen Preparation

Fixation plays an important role in preserving the tissue structures to be visualized using this stain. 10% neutral buffered formalin, or alcoholic formalin fixed, paraffin embedded tissue sections can be used.

BioGenex Alizarin Red Manual Staining Protocol

1. Deparaffinize paraffin sections in xylene and hydrate to water.
2. Rinse the tissue sections in several changes of distilled water.
3. Add 250ul of Alizarin Red S Solution for 2 min, checking microscopically for an orange-red color.
4. Shake off excess dye & blot the sections.
5. Rinse with Acetone or dip [slides](#) in Acetone for 20-30 seconds
6. Add 250ul of Acetone-xylene mix for 20-30 seconds. Shake off excess dye.
7. Repeat step 6 for 2 more times
8. Clear in xylene and mount the slide using permanent mounting medium.

Quality Control

A positive control slide—one that will display positive staining with this stain—should be included in every test. This product has been validated through extensive testing on the following tissue: any calcium containing tissue.

Trouble shooting

1. Follow data sheet instructions correctly.
2. Gently invert all reagents prior to use.
3. pH of Alizarin Red is critical. Adjust the pH of the solution to 4.1-4.3 using 0.5% ammonium hydroxide if there is any deviation

Expected Results

Calcium deposits appear in Orange-Red colour

Limitations of the procedure

1. The thickness of the section may affect the intensity of the staining.
2. Use tissue, which shows positive structure or element for which it is being tested.

Performance Characteristics**Specificity of Special Stain**

The Alizarin Red stain is used to demonstrate calcium in tissue sections.

BioGenex has conducted studies to evaluate the performance of all its Special Stains products. BioGenex Special Stains have shown reproducible and consistent results when used within a single run, between runs and between lots. The products have been determined to be stable for the periods specified on the labels either by standard real time or accelerated testing methods. BioGenex ensures product quality through 100% quality control for all products released and through surveillance programs.

Bibliography

1. Carson F, Histotechnology: A Self-Instructional Text, 1990, pp 226-227, ASCP, Ill.
2. Fredenburgh, JL, Grizzle, WE, Safety and Compliance In the Histology Laboratory: Biohazards to Toxic Chemicals, NSH 1993, Workshop.