

DAHL'S METHOD FOR CALCIUM

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of calcium in tissue.
PRINCIPLE:	Alizarin Red S complexes with calcium in chelate reaction. A counterstain is used to contrast the Alizarin Red S.
CONTROL:	Any known calcium containing tissue <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs010.</i>
SPECIMEN PREPARATION:	Formalin or 95% Reagent Alcohol fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	<p>1. Alizarin Red S 1% Aqueous Item# s2007 <u>Alizarin Red Working Solution:</u> Before using, add 5 mL of Ammonium Hydroxide 0.1% Aqueous per 45 mL of Alizarin Red S 1% Aqueous slowly with constant stirring. Resulting pH should be 6.36-6.40. Solution is stable for one month.</p> <p>2. Ammonium Hydroxide 0.1% Aqueous Item# s2006</p> <p>3. Fast Green Substitute for Light Green 1% Aqueous Item# S230b</p> <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
NOTES:	
REFERENCE:	Luna, Lee G. <u>Histopathologic Methods and Color Atlas of Special Stains and Artifacts</u> . American Histolabs Inc. Gaithersburg, MD 1992. pp. 327-328.

STAINING PROCEDURE:

1. Deparaffinize in 2 changes each of Xylene, Reagent Alcohol, and Reagent Alcohol 95% (Drain off excess 95% Alcohol).
2. Stain in Alizarin Red S Working Solution for 5 minutes.
3. Remove excess stain with distilled water, 5 or 6 rinses.
4. Stain in Fast Green Substitute for Light Green 1% Aqueous for 30 seconds.
5. Rinse in distilled water for 5-10 seconds.
6. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
7. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Calcium Salts Intense Reddish-Orange
Background Pale Green

Poly Scientific R&D Corp.

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