

C-210, DAHL'S METHOD FOR CALCIUM

Fixation: **95% Alcohol** (F-11) or **10% Buffered Neutral Formalin** (F-113)

Sections: Paraffin @ 6 microns

Staining:

1. Deparaffinize and hydrate to **95% Alcohol** (D-11). Drain off excess alcohol.
2. Alizarin Red S Working Solution for two (2) minutes. Remove excess stain with five (5) or six (6) rinses of distilled water.
 To prepare working stain add 10 ml of **Ammonium Hydroxide, 0.1%** slowly with constant stirring, to 100 ml of **Alizarin Red S, 1%** (C-210-1). Resulting pH should be 6.36 - 6.40. Solution is stable for approximately one month.
3. Counterstain with **Light Green Solution** (C-210-2) for one minute. Rinse in distilled water for five to ten (5-10) seconds.
4. Dehydrate in **95% Alcohol** (D-11), **Absolute Alcohol**, (D-10) and clear in **Xylene** (C-120), two changes each.
5. Mount with **Permount** (M-18).

Stain Results:

Calcium Salts	Reddish-Orange
Background	Green.

References:

AFIP Manual of Histological Staining Methods, 3rd ed., Ed. L. Luna: New York: McGraw Hill Publications, c. 1968, p. 175.

Dahl, L. K.: J. Exp. Med., 95:474 (1952).