

TRAP Stain for Paraffin Sections

TECHNIQUE: Formalin fixed, paraffin tissue sections. Mineralized bone MUST be decalcified using EDTA. Acid-decalcifiers inhibit enzymatic staining.

SOLUTIONS:

1. TRAP Basic Incubation Medium (Store at room temp. 6 months)

Sodium Acetate Anhydrous (Sigma S-2889) -----	9.2 g
L-(+) Tartaric Acid (Sigma T-6521) -----	11.4 g
Distilled water -----	950 ml +
Glacial Acetic Acid -----	2.8 ml

Dissolve and adjust pH to 4.7 – 5.0 with 5M Sodium Hydroxide to increase
or more Glacial Acetic Acid to decrease
Bring total volume to 1L with distilled water

2. 5M NaOH (for pH adjustment)

Sodium Hydroxide pellets -----	50 g
Distilled water -----	250 ml

3. Naphthol AS-MX Phosphate Substrate mix

Naphthol AS-MX Phosphate (Sigma N-4875) Store at -20 °C -----	20 mg
Ethylene Glycol Monoethyl Ether (Sigma E-2632) -----	1 ml

Vortex or mix with pipette until dissolved.

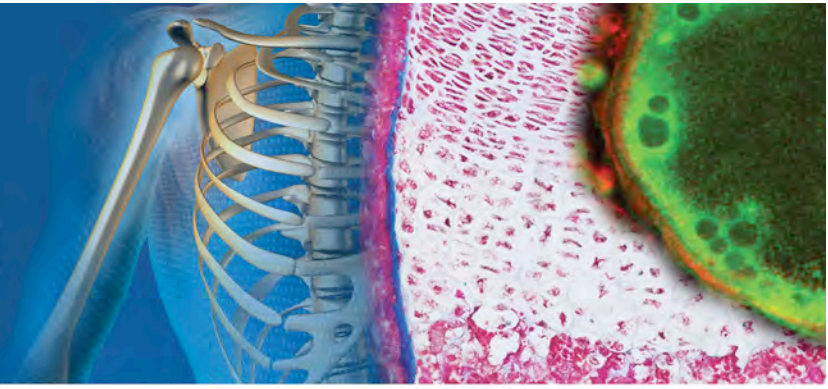
4. TRAP Staining solution Mix (Make fresh each time)

TRAP Basic Incubation Medium-----	200ml
Fast Red Violet LB Salt (Sigma F-3381)-----	120mg
Naphthol AS-MX Phosphate Substrate mix)-----	1 ml

5. 0.02% Fast Green

Fast Green (CAS# 2353-45-9) -----	0.05 g
Distilled water -----	250 ml

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STAINING PROCEDURE:

1. Place TRAP Staining Solution Mix in staining dish and pre-warm to 37°C in waterbath.
2. Deparaffinize slides and rehydrate through graded ethanols to distilled water.
3. Place slides in pre-warmed TRAP Staining Solution Mix and incubate at 37°C for 30 mins. or until control is developed.
4. Rinse in distilled water.
5. Counterstain with 0.02% Fast Green for 30 sec. and rinse quickly in distilled water.
6. Dehydrate quickly through graded alcohols, 5 seconds each, clear in Xylene and mount.

RESULTS:

Osteoclasts ----- red violet
Background ----- green



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