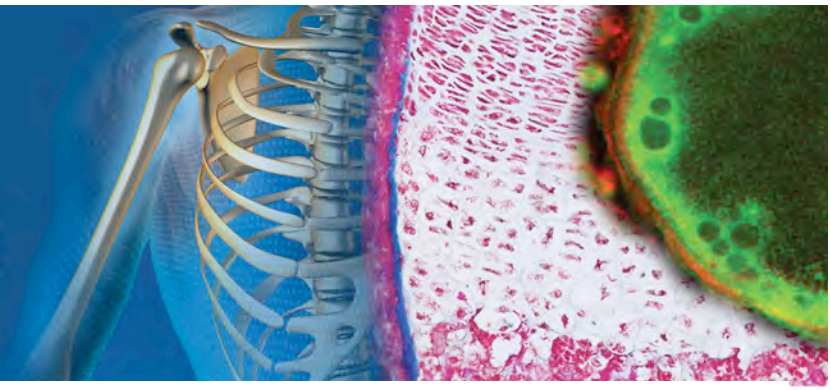


TRAP Stain for Paraffin Sections

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)**

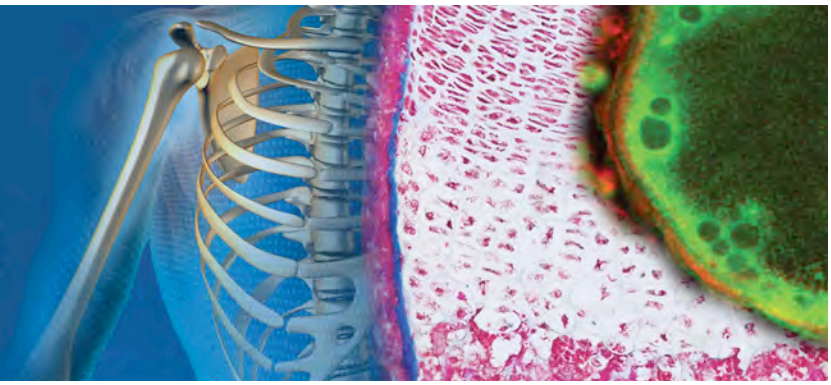
Sodium Hydroxide pellets ----------**50 g**
Distilled water ----------**250 ml**

3. **Naphthol AS-MX Phosphate Substrate mix** (Mix well with vortex/pipette)

Napthol 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**



TRAP Stain for Paraffin Sections (Continued)

5. 0.08% Fast Green

Fast Green (CAS# 2353-45-9) ----------**.02 g**
Distilled water ----------**250 ml**

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 0.08% Fast Green for 1.5 minutes and rinse in several changes of distilled water.
6. Thoroughly air dry slides, dip one by one in Xylene and mount.

RESULTS:

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