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----------------------------------------------- 200 ml
   Fast Red Violet LB Salt ( Sigma F-3381) ------------------------------------ 120 mg
   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
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