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)

   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
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