



TRAP Stain

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

SOLUTIONS:

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

5M NaOH (for pH adjustment)

Sodium Hydroxide pellets ----- 50 g

Distilled water ----- 250 ml

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.

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

0.08% Fast Green (Make fresh each time)

Fast Green (CAS# 2353-45-9) ----- 0.2 g

Distilled water ----- 250 ml

3/2020



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MEDICINE of THE HIGHEST ORDER



TRAP Stain for Paraffin Sections

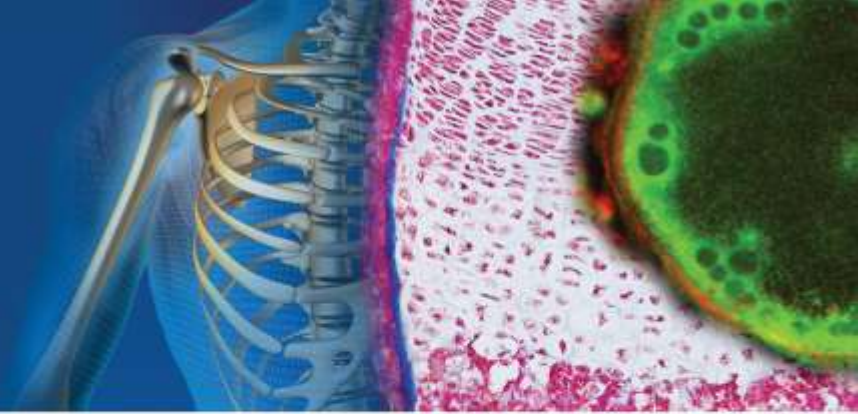
STAINING PROCEDURE:

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 minutes or until control is developed.**
4. Rinse in distilled water.
5. Counterstain with **0.08% Fast Green** for **1.5 minutes**
6. Rinse in **several changes** of distilled water.
7. Thoroughly **air dry slides**
8. Dip one by one in Xylene and mount.

RESULTS:

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

3/2020



TRAP Stain for Frozen Sections

1. Chill 10% Neutral Buffered Formalin (10% NBF) for 20 minutes, **fix samples in cold 10% NBF for 10 minutes.**
2. Rehydrate 5 micron frozen section slides in **1X PBS, 3 times 5 minutes each**
3. Rinse slides in **distilled water, 2 times 1 minute each**
4. Place slides in **pre-warmed TRAP Staining Solution Mix** and incubate at **37°C for 30 minutes** (shorter for thicker sections) or until control is developed
5. Rinse in **distilled water**
6. Counterstain with **0.08% Fast Green for 1.5 minutes**
7. Rinse in **distilled water, 4 times**
8. Thoroughly **air dry slides**
9. Dip **one by one in Xylene and mount.**

RESULTS:

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

3/2020