MICROWAVE P.T.A.H. STAIN KIT PROCEDURE (Phosphotungstic Acid Hematoxylin)

**KIT COMPONENTS INCLUDED:**
- 100ml. FERRIC AMMONIUM SULFATE SOLUTION
- 100ml. P.T.A.H. STAIN
- 1 pint 10% ZINC CHLORIDE SOLUTION

**PRINCIPLE:**
This kit demonstrates collagen, fibrin and striated muscle without using Zenker's Fixative with Mercuric Chloride as a mordant.

**SPECIMEN:**
10% Formalin fixed paraffin embedded tissue cut at 4 to 5 microns.

**EQUIPMENT NOTE:**
Solutions were heated using a 500 watt SAMSUNG microwave oven.

**PROCEDURE:**
1. Deparaffinize slide using Xylene or a Xylene Substitute and hydrate through alcohols to Tap water.
2. Place slide in Distilled water for 1 minute.
3. Put 50ml of 10% ZINC CHLORIDE SOLUTION into a plastic coplin jar and heat in microwave for 20 seconds on high power; remove and stir solution to equalize temperature. Return coplin jar to microwave and heat for 10 seconds on high power; remove and stir solution. Place slide in coplin jar and incubate for 15 minutes.
4. Rinse slide in gently running Tap water for 1 minute.
5. Place slide in Distilled water for 1 minute.
6. Place slide in 25ml of FERRIC AMMONIUM SULFATE SOLUTION, heat in microwave for 10 to 15 seconds on high power, and incubate for 1 to 2 minutes.
7. Rinse slide in gently running Tap water for 4 minutes.
8. Place slide in Distilled water for 1 minute.
9. Heat 25ml of P.T.A.H. STAIN in microwave for 15 to 20 seconds on high power, then remove and agitate to equalize temperature. Place slide into the stain, agitate and incubate for 15 minutes. Heat P.T.A.H. STAIN again for 5 to 10 seconds on high power, agitate slide and incubate for 15 minutes.
10. Differentiate section in 95% Reagent Alcohol. (Check section using microscope for correct differentiation.)
11. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
12. Clear slide through 3 changes of fresh Xylene or Xylene Substitute.
13. Coverslip using a permanent mounting media.

**RESULTS:**
- Collagen: BROWNISH-RED TO RED
- Fibrin, Muscle: BLUE
- Nuclei: BLUE

**REFERENCE:**
Shapiro SH, Sohn LC: Rapid Microwave Phosphotungstic Acid-Hematoxylin Stain for Paraffin and Glycol Methacrylate Sections; The Journal of Histotechnology; Vol 17, No 2, June 1994; 125-126.

For Research Use Only. Not for Diagnostic or Therapeutic Work.

DBS will not be held responsible for patent infringement or other violation that may occur with the use of our product.

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ROOM TEMPERATURE (P.T.A.H.) STAIN KIT PROCEDURE

**PRINCIPLE:** This kit demonstrates collagen, fibrin, and striated muscle without using Zenker's Fixative with Mercuric Chloride as a mordant.

**SPECIMEN:** 10% Formalin fixed paraffin embedded tissue cut at 4 to 5 microns.

**REAGENT NOTE:** Ferric Ammonium Sulfate Solution is not used in this procedure!

**PROCEDURE:**
1. Deparaffinize slide using Xylene or a Xylene Substitute and hydrate through alcohols to Tap water.
2. Place slide in Distilled water for 1 minute.
3. Place slide in room temperature **10% Zinc Chloride Solution** for 3 hours.
4. Rinse slide thoroughly in running Tap water.
5. Place slide in Distilled water for 1 minute.
6. Place slide in room temperature **P.T.A.H. Stain** for 12 to 24 hours with periodic agitation.
7. Differentiate section in 95% Reagent Alcohol. (*Check section using microscope for correct differentiation.*)
8. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
9. Clear slide through 3 changes of fresh Xylene or Xylene Substitute.
10. Coverslip using a permanent mounting media.

**RESULTS:**
- Nuclei: **BLUE**
- Fibrin, Muscle: **BLUE**
- Collagen: **BROWNISH RED TO RED**


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