PTAH Stain Kit
(Phosphotungstic Acid Hematoxylin)

Description: The PTAH Stain Kit for Microwave is intended for use in the histological visualization of collagen, striated muscle, glial fibers and collagen without using Zenker’s Fixative with Mercuric Chloride as a mordant. This kit may be used on formalin-fixed, paraffin-embedded or frozen sections.

Uses/Limitations: For In-Vitro Diagnostic use only. Histological applications. Do not use past expiration date. Use caution when handling these reagents.

Control Tissue: Striated Muscle

Results:

Fibrin, Striated Muscle, Glial Fibers: Blue
Collagen: Brownish/Red
Nuclei: Blue

Kit Contents:

<table>
<thead>
<tr>
<th>Item #</th>
<th>Kit Contents</th>
<th>Volume</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC-ZCS500</td>
<td>Zinc Chloride Solution (10%)</td>
<td>500 ml</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>SSC-FAS125</td>
<td>Ferric Ammonium Sulfate</td>
<td>125 ml</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>SSC-HPA125</td>
<td>PTAH Solution</td>
<td>125 ml</td>
<td>Room Temperature</td>
</tr>
</tbody>
</table>

For information regarding ordering individual components, please contact us at: 800-442-3573.

Precautions: Avoid contact with skin and eyes.
Harmful if swallowed.
Follow all Federal, State, and local regulations regarding disposal.
Use in chemical fume hood whenever possible.
Procedure (60° C. Water Bath):

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Pour Zinc Chloride Solution (10%) into plastic staining jar and set in 60° C. water bath for 10 minutes to equilibrate temperature.
3. Place slide in warmed Zinc Chloride Solution (10%) and incubate for 20 minutes at 60°C.
4. During step 3, pour Ferric Ammonium Sulfate Aqueous Solution into a second plastic staining jar and set in 60° C. water bath for 10 minutes to equilibrate temperature.
5. Rinse slide in running tap water for 1 minute.
6. Rinse in distilled water for 1 minute.
7. Place slide in warmed Ferric Ammonium Sulfate Aqueous Solution and incubate for 5 minutes at 60°C.
8. During step 7, pour Phosphotungstic Acid Hematoxylin Solution into a third plastic staining jar and set in 60° C. water bath for 10 minutes to equilibrate temperature.
9. Rinse slide in running tap water for 2 minutes.
10. Rinse in distilled water for 1 minute.
11. Place slide in warmed Phosphotungstic Acid Hematoxylin Solution and incubate for 60 minutes at 60°C.
12. Differentiate section in 95% Reagent Alcohol. Check section using microscope for proper differentiation.

Note: Graded alcohols will remove some stain.

13. Dehydrate in 3 changes of Absolute Alcohol.
14. Clear in 3 changes of fresh Xylene or Xylene Substitute, and mount in synthetic resin.

Procedure (Microwave):

Equipment Needed: 500 Watt Microwave Oven

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place slide to fresh distilled water for 1 minute.
3. Pour 50 ml of Zinc Chloride Solution (10%) into plastic coplin jar and heat in microwave for 20 seconds on high power. Remove jar and stir solution to equalize temperature. Return coplin jar to microwave and heat for 10 seconds on high power. Remove jar and stir solution to equalize temperature.
4. Place slide in coplin jar and incubate for 15 minutes.
5. Rinse slide in running tap water for 1 minute.
6. Rinse in distilled water for 1 minute.
7. Place slide in 25 ml Ferric Ammonium Sulfate Aqueous Solution, heat in microwave for 15 seconds on high power and incubate for 2 minutes.
8. Rinse slide in running tap water for 2 minutes.
9. Rinse in distilled water for 1 minute.
10. Heat 25 ml Phosphotungstic Acid Hematoxylin Solution in microwave for 20 seconds on high power. Remove and agitate to equalize temperature of solution. Place slide in stain, agitate and incubate for 15 minutes. Reheat solution for 10 seconds on high power, agitate and incubate for another 15 minutes.

11. Differentiate section in 95% Reagent Alcohol. Check section using microscope for proper differentiation.

12. Dehydrate in 3 changes of Absolute Alcohol.

13. Clear in 3 changes of fresh Xylene or Xylene Substitute, and mount in synthetic resin.

References:

Shapiro, S.H., Sohn, L.C.: Rapid Microwave Phosphotungstic Acid-Hematoxylin Stain for Paraffin and Glycol Methacrylate Sections; The Journal of Histotechnology; Volume 17, Number 2, June 1994, pages 125-126.
Lot-to-Lot Validation Form
PTAH Stain Kit Catalog: SSK-PTAH

Kit Lot Number: __________________________
Kit Expiration Date: __________________________
Date Tested: __________________________
Control Tissue (#): __________________________
Approved for Use: Y/N __________________________
Date put into use: __________________________

If not approved, corrective actions taken: __________________________
Approved by: __________________________

<table>
<thead>
<tr>
<th>Kit Component</th>
<th>Lot #</th>
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<tbody>
<tr>
<td>Zinc Chloride Sol (10%)</td>
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<tr>
<td>Ferric Ammonium Sulfate</td>
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<tr>
<td>PTAH Solution</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Replacement Component if used</th>
<th>Replacement Date</th>
<th>Lot #</th>
<th>Accepted Y/N</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>Zinc Chloride (10%)</td>
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<tr>
<td>Ferric Ammonium Sulfate</td>
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<tr>
<td>PTAH Solution</td>
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Approved By: __________________________

StatLab is providing this form to assist with reagent lot validation as stated in CLIA’88 Standard 493.1256-For reagent(s), the laboratory must do the following: Check each batch (prepared in-house), lot number (commercially prepared) and shipment of reagents, stains, and identification systems (systems using two or more substrates or two or more reagents, or a combination) when prepared or opened for positive and negative reactivity, if applicable.