INTENDED USE AND PRESENTATION:
For in vitro diagnostic use.
AP0507. 100 test.

APPLICATIONS:
The PTAH-Phosphotungstic Acid Hematoxylin kit was designed initially to stain neuroglia but at present it is used especially to differentiate between smooth muscular tissue and striated muscular tissue, since it stains isotropic bands of skeletal muscle’s myofibrils. Moreover, it is one of the recommended methods to show fibrin.
The interpretation of the stain results is the full responsibility of the user. Any experimental result must be confirmed by a medically established diagnostic product or procedure.

PRODUCT COMPOSITION:
The PTAH-Phosphotungstic Acid Hematoxylin kit is composed by:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Name</th>
<th>Volume</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Potassium permanganate solution</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
<tr>
<td>B</td>
<td>Acid buffer</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
<tr>
<td>C</td>
<td>Oxalic acid solution</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
<tr>
<td>D</td>
<td>P.T.A.H. according to Mallory solution</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
</tbody>
</table>

METHODS AND PROCEDURE:
Principles of the method: Phosphotungstic acid acts as an acid carrier for the dye; it attaches to fibrin and to tissue elements with both chemical and physical links. Unlike most hematoxylin, this is a “progressive” method.
Specimen: Paraffin-embodied tissue samples should be used.
Procedure time: 10 minutes + overnight.
Staining procedure:
1) Deparaffinize and hydrate for paraffin section.
2) Put the slide in distilled water.
3) In a separate tube, mix equal parts of Reagent A and Reagent B, put on the section and leave to act 5 minutes.
4) Wash in distilled water.
5) Put on the section 2-5 drops of Reagent C and leave to act 5 minutes.
6) Wash in distilled water.
7) Put on the section 10 drops of Reagent D, close the container and incubate the slide overnight.
8) Rinse quickly in distilled water (3-4 seconds).
9) Dehydrate in alcohols of increasing clear in xylene and mount preparation.
See our web site at www.gennova-europe.com for detailed protocols ancillary reagents and support products.

EXPECTED RESULTS:
During the reaction of the different solutions of the kit with the tissue sections, resulting in the following stains:

<table>
<thead>
<tr>
<th>Component</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei, fibrins (for a great part) myofibrils, astrocytes, some elastic fibers, neuroglia, myelin fibers</td>
<td>Dark blue</td>
</tr>
<tr>
<td>Collagen, bone matrix, cartilage</td>
<td>Brick red in various tonalities</td>
</tr>
</tbody>
</table>

REQUIRED MATERIALS BUT NOT SUPPLIED:
All reagents, materials, and laboratory equipment for this procedure are not provided with this kit. This includes adhesive slides and cover slips, positive and negative control tissues, xylene or adequate substitute, ethanol, distilled H2O, pipettes, Coplin jars, glass jars, moist chamber, histological baths, mounting materials, and microscope.
Buffered solutions for and other auxiliary reagents are available from Gennova Scientific.

STORAGE AND STABILITY:
Store at 15-25 °C until the expiration date printed on product label. Do not use after the expiration date. Keep the containers tightly closed. After the first opening, the product is usable until the expiry date stated on label of intact product. Waste from solutions must be disposed under the procedure of hazardous substance. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user.
Gennova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances is Gennova Scientific obliged to cover damages caused by use of this reagent.
TROUBLESHOOTING:
If unusual staining is observed or any other deviations from the expected results, please read these instructions carefully, and if this does not solve the problem, please contact Gennova Scientific’s technical support department or your local distributor.

PRECAUTIONS:
Use only by qualified personnel. Read with attention the information written on the label (dangerous symbols, risks and safety phrases). Consult always the safety data sheet (MSDS) where the information about the risks of the preparation, precautionary measures during use, first aid and disposal are available. Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. MSDS is available upon request.

PERFORMANCE CHARACTERISTICS:
Gennova Scientific has performed studies to evaluate the functioning of this product, concluding that the product is both specific and sensitive for recommended use.

BIBLIOGRAPHY:
Peers J.H. A modification of Mallory’s phosphotungstic acid hematoxylin stain for formaldehyde-fixed tissue. Arch Path 1941; 32: 446-449.