INTENDED USE AND PRESENTATION:
For in vitro diagnostic use.
AP0351. 100 Tests.

APPLICATIONS:
The AfoG (Acid Fuchsin Orange G) kit was designed to show glomerular protein deposits in kidney biopsy. 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 AfoG (Acid Fuchsin Orange G) 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>Weigert’s iron hematoxylin A</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
<tr>
<td>B</td>
<td>Weigert’s iron hematoxylin B</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
<tr>
<td>C</td>
<td>Phosphomolybdic acid solution</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
<tr>
<td>D</td>
<td>AfoG solution</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
</tbody>
</table>

METHODS AND PROCEDURE:
Principles of the method: In this method 3 different dyes are used: Weigert hematoxylin for nuclear staining, orange G for cytoplasm and aniline blue for a selective collagen staining. Selectivity in this procedure is due to different degrees of affinity between dyes and tissue macromolecules. A central role is played by phosphomolybdic acid which acts as a bound between tissue structures (collagen fibrils, cell membranes) and aniline blue (amphoteric dye). Orange G, which is the second component of AFOG solution, has no affinity to phosphomolybdic acid and is thus used to stain all remaining structures. Acid fuchsin stains selectively glomerular protein deposits. Specimen: Paraffin-embedded tissue samples should be used.
Procedure time: 30 minutes.

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 and leave under section 10 minutes.
4) Wash in running water for 5 minutes.
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 2-5 drops of Reagent D and leave to act 5 minutes.
8) Wash in distilled water.
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>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen fibrils</td>
<td>Blue</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Black</td>
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<tr>
<td>Erythrocytes, cytoplasm</td>
<td>Pink-orange</td>
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<tr>
<td>Elastic fibrils</td>
<td>Pale pink or unstained</td>
</tr>
<tr>
<td>Glomerular protein deposits</td>
<td>Lively red</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

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**Afog (Acid Fuchsin Orange G), Kit**
Reference: AP0351

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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: