INTENDED USE AND PRESENTATION:
For in vitro diagnostic use.
AP0267. 100 Tests.

APPLICATIONS:
The Masson Trichrome Goldner (with light green) kit is a recommended method for connective tissue. It demonstrates gametes, nuclei, neurofibris, neuroglia, collagen, keratin, intracellular fibrils and negative image of Golgi apparatus. It is very suitable for black and white microphotographs.
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 Masson Trichrome Goldner (with light green) 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>Biebrich scarlet-acid fuchsin solution</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
<tr>
<td>D</td>
<td>Phosphotungstic acid Mallory solution</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
<tr>
<td>E</td>
<td>Light green solution</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
<tr>
<td>F</td>
<td>Bouin solution</td>
<td>30 mL</td>
<td>15-25 °C</td>
</tr>
</tbody>
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METHODS AND PROCEDURE:

Principles of the method: Three different stains are used: Weigert’s iron hematoxylin for nuclei, a mixture of acid dyes (Biebrich scarlet-acid fuchsin) for cytoplasm and light green for collagen.

Specimen: Paraffin-embedded tissue samples should be used.

Procedure time: 40 minutes.
Prepare the following solution before use:
-  Working Weigert’s iron hematoxylin Solution: Mix Reagent A and Reagent B in equal parts. (Please note after reconstitution solution can be used for maximum two weeks).

Staining procedure:
1) Deparaffinize and hydrate to distilled water. Treat with Bouin (Reagent F) if sections are formalin fixed for one hour at 56° C or overnight at room temperature. Let stand for 10 minutes to cool. Omit step if it was initially fixed in Bouin.
2) Wash in running water until sections are clear.
3) Rinse in distilled water.
4) Stain in Working Weigert’s iron Hematoxylin Solution for 10 minutes.
5) Wash in running water for 2-5 minutes.
6) Wash in distilled water.
7) Stain in Reagent C for 15 minutes.
8) Rinse in distilled water.
9) Differentiate in Reagent D for 10 minutes (Check to see that collagen is not red, repeat differentiation if collagen retains the red colour).
10) Counterstain in Reagent E for 1 minute.
11) Rinse quickly in acetic water or alcohol acid.
12) Dehydrate in alcohols of increasing clear in xylene and mount.

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:

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Nuclei</td>
<td>Black</td>
</tr>
<tr>
<td>Muscle, cytoplasm and keratin</td>
<td>Red</td>
</tr>
<tr>
<td>Collagen</td>
<td>Green</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:**
Lillie RD. Further experiments with the Masson trichrome modification of Mallory’s connective tissue stain. Stain Technol 1940; 15:82.