Haematoxylin (Mayer's) is recommended for Immunohistochemical and cytochemical Staining (as Nuclear Counter Stain) (PAS Staining Procedure). It may also be used for routine Haematoxylin and Eosin Staining.

**Composition**

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium potassium Sulphate</td>
<td>50.00 gm</td>
</tr>
<tr>
<td>Sodium Iodide</td>
<td>0.20 gm</td>
</tr>
<tr>
<td>Haematoxylin</td>
<td>1.00 gm</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>20,000 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>960,000 ml</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

**Directions**

A. Nuclear counter stain for Immunohistochemical staining:

1. Complete individual staining procedure (as desired). Rinse the slide with deionized water.
2. Stain the tissue section or the cell preparation with haematoxylin for 30-60 seconds.
3. Rinse with water to remove excess reagent.
4. Place in bluing reagent (alkaline solution such as a weak ammonia solution, 0.08% in water) until stain is blue (approximately 30 seconds).
5. Rinse in deionized water.
6. Section can be mounted in aqueous mounting media.

B. Hematoxylin and Eosin staining:

1. Prepare 95% alcohol solution.
2. Deparaffinize the tissue section and hydrate to water or fix and hydrate frozen sections.
3. Stain tissue section or cell preparation for 30-60 seconds with haematoxylin.
4. Rinse with water to remove excess reagent.
5. Place in bluing reagent until the stain is blue.
6. Rinse in deionized water.
7. If alcoholic eosin is used, place slide in 95% alcohol for 30 seconds.
8. Place eosin counter stain for 30-60 seconds.
9. Dehydrate in two changes each of reagent 95% alcohol, absolute alcohol and xylene for 2 minutes each.
10. Mount with synthetic mounting medium and examine the slide under microscope.

**Principle And Interpretation**

Haematoxylin is extracted from logwood with hot water, and then precipitated out from the aqueous solution using urea. Haematoxylin itself is not a stain. Its major oxidation product is Hematein that is a natural dye responsible for colour properties. Hematein can be produced by natural oxidation on exposure to light and air. Ehrlichs and Delafields hematoxylin solutions are examples of naturally ripened hematoxylin. Chemical oxidation uses sodium iodate (eg., Mayers hematoxylin) or mercuric oxide (eg., Harris hematoxylin). Hematein is anionic, having poor affinity for tissue, and is inadequate as a nuclear stain without the presence of a mordant. The mordants used are salts of aluminium, iron, tungsten. Mayers hematoxylin is alum hematoxylin, chemically ripened with sodium iodate. It can be used as a regressive stain like any alum hematoxylin. However it is also useful as a progressive stain, particularly in situations where a nuclear counterstain is needed in the demonstration of glycogen, in various enzyme histochemical techniques (1). Haematoxylin and eosin are the principle stains used for the demonstration of nucleus and the cytoplasmic inclusions. Here, acid reacting components of the cell combine with alkaline dyes and the alkaline area react with acid dyes.
**Type of specimen**
Clinical samples: Blood sample

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines(1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**
In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations**
1) To preserve morphology of cells, films must be fixed without delay and the films should never be left unfixed for more than a few hours.
2) Methanol used as fixative should be completely water free. As little as 1% water may affect the appearance of the films and a higher water content causes gross changes.
3) The red cells will also be affected by traces of detergent on inadequately washed slides.
4) Sometimes when thick films are stained they become overlaid by a residue of stain or spoil by the envelopes of the lysed red cells.

**Performance and Evaluation**
Performance of the stain is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

**Quality Control**

**Appearance**
Red coloured solution.

**Clarity**
Clear without any particles.

**Microscopic Examination**
Staining is carried out staining characteristics is observed under microscope by using oil immersion lens.

**Results**
Nuclei: Blue

**Storage and Shelf Life**
Store between 10 - 30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

**Disposal**
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

**Reference**
5. Staining Procedures;Fourth Edition ;Williams& Wilkins;Baltimore
Disclaimer:

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