



## Haematoxylin (Ehrlich)

S059

### Intended use

Haematoxylin (Ehrlich) is used for nuclear stain for Immunohistochemical and cytochemical staining. It may also be used for the routine Hematoxylin and Eosin staining.

### Composition\*\*

#### Ingredients

Haematoxylin	2.0 gm
Alcohol 95%	100.0 ml
Glycerine	100.0 ml
Aluminium potassium sulfate (Alum)	3.0 gm
Glacial acetic acid	10.0 ml
Sodium iodate	0.10 gm
Distilled water	100.0 ml

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

### Directions

#### A. Hematoxylin staining (H&E staining):

1. Flame the slide and place in xylene for 3-4 minutes. Repeat xylene treatment with agitation.
2. Dip in 100% absolute alcohol for 30-60 seconds. Next dip in 90%, 80% and in 70% absolute alcohol. Wash in tap water and rinse in distilled water.
3. Stain tissue section or cell preparation for 2-5 min. with hematoxylin (Ehrlich) (S059).
4. Rinse with water to remove excess reagent.
5. Place in 0.5%(V/V) Hydrochloric acid
6. Rinse in distilled water for 30-60 seconds
7. Dip in dilute ammonia water till section appears blue.
8. Wash in tap water and place slide in 95% alcohol for 30 sec.
9. Place eosin counter stain for 30-60 sec. Drain the solution 10. Dip slide in 70% alcohol for 30-60 seconds.
11. Place in 95% alcohol for 30-60 seconds.
12. Place in absolute alcohol (2 changes, 30-60 seconds each).
13. Place the slide twice in xylene for 30-60 seconds.
14. Drain excess xylene and mount on DPX or Canada balsam with a coverslip.

The first 2 steps of the procedure are collectively referred to in all staining procedures as 'deparaffinize.' Steps 3-9 are referred to as 'staining'. The last 5 steps are referred to in all staining methods as 'dehydrate, clear, and mount.'

#### B. Hematoxylin Nuclear counter staining:

1. Deparaffinize the section.
2. Carry out the individual staining procedure (as desired).
3. Rinse the slide with deionized water.
4. Counter stain the tissue section with hematoxylin (Ehrlich) (S059) for 2-5 min.

5. Rinse with water to remove excess reagent.
6. Place in bluing reagent (alkaline solution such as a weak ammonia solution, 0.08% in water) until stain is blue (approximately 30 sec.).
7. Rinse in distilled water.
8. Section can be mounted in aqueous mounting media.

## Principle And Interpretation

Hematoxylin is extracted from the heartwood of the logwood tree, Hemtoxylin campechianum. Hematoxylin (Ehrlich) solution contains the dye, hematin and the Aluminum potassium sulfate as a mordant which provides the stain colour (blue) glacial acetic acid controls the pH of the Solution. It can be used as nuclear counter stain (as in PAS staining etc.) Hematoxylin (Ehrlich) reagent is suitable for immunohisto chemical application. It is the slowest of progressive stains. For histochemical purposes, the progressive staining is commonly used in which dye selectively stains the nuclear chromatin without staining cytoplasmic structures. Slides are left in the hematoxylin solution only long enough to stain the nuclei. The excess dye should be removed by 'blueing' of the tissue. Initially the tissue sections are coloured either purple or reddish purple, on exposure to alkaline solution, the tissue section takes on the characteristic blue colour. Hematoxylin-Eosin is the commonly used stain, which is specific for certain substances of diagnostic importance. Here, acid reacting components of the cell combine with alkaline dyes and the alkaline area react with acid dyes. The stain is available for amyloid, lipids, inorganic substances such as iron and calcium, pigments like melanin and hemosiderin, carbohydrates and mucopolysaccharides.

## Type of specimen

Clinical samples: Blood sample

## Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines. 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 medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

## Quality Control

- **Appearance** : Wine red coloured solution.
- **Clarity** : Clear without any particles.
- **Microscopic Examination** : Immunohistochemical / cytochemical staining is carried out and staining characteristic is observed under microscope.
- **Results** : A) Haematoxylin Stainig (H&E staining)
  - Nuclei : Blue colour
  - Cytoplasm : Pink colourB) Haematoxylin Nuclear counter staining:
  - Nuclei: Blue colour

## 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

## Reference

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5. Staining Procedures; Fourth Edition; Williams& Wilkins; Baltimore
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Storage temperature



Do not use if package is damaged



In vitro diagnostic medical device



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