



Haematoxylin (Ehrlich)

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	2.0gm
Alcohol 95%	100.0ml
Glycerine	100.0ml
Aluminium potassium sulfate (Alum)	3.0gm
Glacial acetic acid	10.0ml
Sodium iodate	0.1gm
Distilled water	100.0ml

**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 2-5min. with hematoxylin (Ehrlich) (S059).
4. Rinse with water to remove excess reagent.
5. Place in 0.5% (9V/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.

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 colour

B)Haematoxylin Nuclear counter staining:

Nuclei : Blue colour

Reference

- 1) Clark G (Ed) 1981, Staining Procedures, 4th edition, Published for the Biological Stain Commission by Williams & Wilkins, London.
- 2)Bauer J.D., Ackermann P.G. and Toro G. (Eds.), 1974, Clinical Laboratory Methods, 8th ed., The C.V. Mosby Co., St. Louis.
- 3) Ehrlich, P. 1886. Die von mir herrührende Hämatoxylinlösung. Z. Wiss. Mikrosk. 3: 150

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.