H & E Stain

Intended use
H & E Staining method is used for the routine staining of the cationic and anionic tissue components in tissue sections. This is the standard reference stain used in the study of histochemical tissue pathology.

Summary
Hematoxylin and eosin stain (abbreviated as H & E stain) is one of the principle tissue stains used in routine histology staining methods. It is the most widely used stain in medical diagnosis and is often the gold standard; wherein, when a pathologist looks at a biopsy of a suspected cancer, the histological section is likely to be stained with H & E.
H & E Stain is the combination of two histological stains: Hematoxylin and Eosin. The hematoxylin is a selective nuclear stain which stains the cell nuclei blue, and eosin stains the extracellular matrix and cytoplasm pink, with other structures taking on different shades, hues, and combinations of these colors. The stain shows the general layout and distribution of cells and provides a general overview of a tissue sample's structure. Hence, there is a clear differentiation between the nuclear and cytoplasmic parts of a cell.

Principle
Hematoxylin and eosin are the principle stains used for the demonstration of nucleus and the cytoplasmic inclusions. Alum acts as a mordant and hematoxylin containing alum stains nucleus light blue which turns red in the presence of acid. The cell differentiation is achieved by treating the tissue with acid solution. Counter staining is performed by using eosin solution which imparts pink color to the cytoplasm.
Hematoxylin, a common nuclear stain, is isolated from an extract of logwood (*Haematoxylon campechianum*). Before hematoxylin can be used as a nuclear stain, it must be oxidized to hematein and combined with a metallic ion (mordant). Most successful mordants have been salts of aluminum or iron. Generally, hematoxylins are classified as progressive or regressive based on dye concentration. Progressive stains (e.g., Mayer's hematoxylin) have a lower concentration of dye and selectively stain nuclear chromatin. The desired intensity is a function of time. Regressive stains (e.g., Harris hematoxylin) color all nuclear and cytoplasmic structures intensely. To arrive at correct chromatic response, excess dye must be removed by treatment with dilute acid (differentiation).
Eosin is tetra bromofluorescein (a substituted xanthene), a red acidic dye and fluorochrome. The dye is very soluble in ethyl alcohol and also used for the staining of cytoplasm. Eosin Y is the most commonly used counterstain for hematoxylin.

Reagents / Contents
1. Hematoxylin Harris

Hematoxylin	5.0 g
Ammonium/ Potassium Alum	100 g
Mercuric Oxide	2.5 g
Alcohol 95%	50 mL
Distilled Water	1000 mL

Appearance: Maroon purplish solution.

2. Eosin (AQU.) 2%

Eosin-Y	2.0 g
Distilled water	100 mL

Appearance: Dark reddish solution.

Storage and Stability
Store at 15°C-30°C away from bright light. Use before expiry date on label.

Materials required but not provided
Tissue section specimen on clean grease-free glass slide, staining rack, blotting paper, immersion oil (Cat. No. 207090110025) and microscope. Reagents and solutions required but not provided in the Kit such as xylene, a

series of descending and of ascending grades of alcohol, 1% acid alcohol solution, Scott's Tap Water Buffer (Cat. No. 207191390035) and DPX mountant (Cat. No. 207040250100).

Type of Specimen

Histochemical tissues sections obtained from biopsy specimens.

Procedure

1. Sections are deparaffinized (removal of wax) by placing in xylene for 10 - 15 minutes.
2. Rehydrate section by passing in a series of descending grades of alcohol (100%, 95%), finally to water.
3. Place in Hematoxylin Harris solution for 8-10 minutes.
4. Rinse in water.
5. Differentiate the slide in a solution 1% acid alcohol for 10 seconds.
6. Rinse in tap water.
7. Blueing (brining the required blue color to section) is done by putting the section in a solution containing Sodium bicarbonate, $MgSO_4$ and saturated solution of Lithium carbonate (Scott's Tap Water Buffer, Cat. No. 207191390035) for 6 minutes.
8. Counter stain with aqueous Eosin (Aqu.) 2% for 3 minutes.
9. Rinse in tap water.
10. Sections are dehydrated which is done by a series of ascending grades of alcohol (95%, 100%) and finally clearing in Xylene.
11. Dry the section by pressing on the filter paper.
12. Mount in DPX mountant (Cat. No. 207040250100) and observe under microscope, 40X and 100X under oil immersion lens.

Interpretation of Results

The nuclei of cells are stained blue or dark-purple along with a few other tissues, such as keratohyalin granules and calcified material with Hematoxylin. The cytoplasm and some other structures including extracellular matrix such as collagen stains in up to five shades of pink with Eosin. Most of the cytoplasm is eosinophilic and is rendered pink. Red blood cells are stained intensely red. The background of the tissue remains colorless.

Warranty

H & E staining solutions are for "*In Vitro* Diagnostic Use" only. This product is designed to perform as described on the label and pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation

Cat No.	Product	Pack Size
207080190125	H & E Stain Kit	2 x 125 mL
207080190250	H & E Stain Kit	2 x 250 mL
207050320125	Eosin (AQU.) 2%	125 mL
207050320250		250 mL
207050320500		500 mL
207050325000		On request
207080070125	Hematoxylin Harris	125 mL
207080070250		250mL
207080070500		500 mL
207080075000		On request

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.