

HEMATOXYLIN-EOSIN STAIN

Principle

Routine staining method in histology and cytology. It is a staining based on two stages, the first a nuclear staining by a basic dye (hematoxylin) and the second, a cytoplasmic staining by an acid xanthenic dye (eosin).

Hematoxylin in combination with aluminum, iron or chromium salts forms an active dye, hematein, formed by the oxidation of hematoxylin. This is used as a nuclear dye, staining the blue / black cores and providing a good detail of them. For this reason, it is often used together with a cytoplasmic dye, usually eosin, which contributes a gradation between pink and red to cationic character structures and matrices (to which hematoxylin does not stain or do very weakly). This gives a good contrast of the microscopic preparations facilitating their observation.

Material

Paraffin cuts, frozen cuts, clinical cytological material.

Reagents

Code	Description
251299	Eosin Yellowish (C.I. 45380) for clinical diagnosis (*)
256879	Eosin Yellowish alcoholic solution 1% for clinical diagnosis (*)
251301	Eosin Yellowish hydroalcoholic solution 1% for clinical diagnosis (*)
255298	Carazzi's Hematoxylin solution for clinical diagnosis (*)
253949	Harris Hematoxylin solution for clinical diagnosis (*)
256991	Harris Hematoxylin modified solution for clinical diagnosis (*)
252081	Phloxine B (C.I. 45410) for clinical diagnosis
251008	Acetic Acid glacial for clinical diagnosis
251769	Xylene, mixture of isomers for clinical diagnosis (*)
192695	Ethanol 70% v/v (BP) pharma grade
251085	Ethanol 96% v/v for clinical diagnosis (*)
251086	Ethanol absolute for clinical diagnosis (*)
253681	Eukitt [®] , mounting medium for clinical diagnosis

Preparation of solutions

- Eosin stock solution: Dissolve 1.0 g Yellowish Eosin (C.I. 45380) in water and make up to 100.0 ml with water.
- Floxin stock solution: Dissolve 1.0 g Floxin B (C.I. 45410) in water and make up to 100.0 ml with water.
- Eosin-floxin mother solution: According to Eosin's presentation to be used, prepare the following. Mix the components and homogenize well and keep protected from light.

1. With Yellow Eosin (C.I. 45380)

Eosin Stock solution	10 ml
Phloxine Stock solution	1 ml
Ethanol 96%	78 ml
Acetic acid glacial	0.4 ml

2. With Eosin Yellowish hydroalcoholic solution 1%

Eosin Yellowish hydroalcoholic solution 1%	10 ml
Phloxine Stock solution	1 ml
Ethanol 96%	78 ml
Acetic acid glacial	0.4 ml

3. With Eosin Yellowish alcoholic solution 1%

Eosin Yellowish solution	10 ml
Phloxine Stock solution	1 ml
Water	9 ml

Procedure

STAGE	REAGENT	TIME
Dewax	Xylene	3 x 5 min
Hydrate	Ethanol absolute	7 min
	Ethanol 90 %	7 min
	Ethanol 70 %	7 min
	Distilled water	7 min
Stain	If Hematoxylin Harris or Harris Modified	6 min
	If Hematoxylin Carazzi	6-9 min depending on sample size
Clean	Running water	5 min
Turn staining ⁽¹⁾	Acetic acid 2 %	10 – 20 s
Clean	Running water	5 min
	Ethanol 96 %	1 min
Stain	Eosin-Ploxine Solution	5 min
Dehydrate	Ethanol 70 %	5 s
	Ethanol 90 %	5 s
	Ethanol absolute	1 min
	Ethanol absolute	5 min
Clear	Xylene	5 s + 5 min drying
	Xylene	5 min + drying
Mounting	Mounting medium	

⁽¹⁾ If Carazzi Hematoxylin is used skip this step.

Results

	Harris Hematoxylin	Harris Hematoxylin modified	Carazzi's Hematoxylin
Nuclei	Intense bluish	Bluish	Light blue-violet
Cytoplasm and matrix	Different shades of Pink to violet.	Different shades Pink and reddish.	Different pink tones

The tonality of the results will differ according to the Hematoxylin used and not according to the Eosin.

Technical note

Filter any soil present in the solution. The microscope used should correspond to the requirements of a clinical diagnostic laboratory. If an automatic staining device is used, the operating instructions of the appliance manufacturer and the software must be observed.

Sample preparation

All samples should be treated according to the state of the technology. All samples must be unambiguously labeled.

Diagnostics

Diagnosis should be established only by authorized and qualified persons. Each application should involve appropriate controls to rule out erroneous results.

Storage

The staining solution should be stored at room temperature.

Expiration

The product stored at the indicated temperature and in a tightly closed container is usable until the expiration date indicated on the package.

Notes on use

To avoid errors, the staining must be carried out by specialized personnel. For professional use only. The national directives on safety at work and quality assurance must be complied with.

Advise on disposal of waste

Solutions used and expired solutions should be disposed of as hazardous waste and local waste disposal regulations must be observed. If further questions are asked about disposal, they may be processed through E-Mail: info.es@itwreagents.com. Inside the EU are valid the requirements based on Council Directive 67/548/EEC on the approximation of the laws, regulations and laws, regulations and administrative provisions relating to the classification, packaging and labeling of dangerous substances in the relevant version.

Classification of hazardous substances

Observe the classification of dangerous substances on the label and the information on the safety data sheet.

Manufacturer

Panreac Química S.L.U.

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(*) Sanitary product for In Vitro Diagnostics

