

Histology Core Facility
BIOENG-518
2025

Hematoxylin and Eosine on frozen sections

H&E - cryo

PURPOSE: In histology, the standard or "routine stain" is the hematoxylin and eosin (erythrosine) stain, better known as the "H&E" stain. This stain is a simple method for general morphology of the tissue.

PRINCIPLE: The H&E stain uses two separate dyes. Hematein (oxidation product of hematoxylin) has basic properties which enables it to interact with the rather acidic DNA located in the nuclei and gives it a blue staining. Eosine, an acid chemical, stains basic cells structures like plasma proteins in pink.

FIXATIVE: 4% Buffered PFA, 10% formalin, or any other fixatives.

SECTIONS: Paraffin and frozen sections.

CONTROL: All healthy tissues

METHOD

- | | |
|--------------------------------|---------|
| 1. Air dry at room temperature | 15 min |
| 2. Fixation with 10% Formalin | 10min |
| 3. Wash in PBS 1x | 3x 2min |

PRISMA automate

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|--|-------------|------------------|
| 4. Place the sections in Harris Hematoxylin (Gill II PAP 1) | 1 min | nuclear staining |
| 5. Wash in tap water | | |
| 6. Differentiate in 1% acid-alcohol | few seconds | |
| 7. Wash in running tap water
<small>A blueing solution can replace 10min in tap water</small> | 10 min | nuclear blueing |
| 6. Working solution Eosine-Phloxine
<small>staining</small> | 1 min | cytoplasm |
| 7. Wash briefly in water | | |
| 8. Dehydrate, clear and mount (Eukitt) | | |
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RESULTS

Nuclei	Blue-black
Cytoplasm	Varying shades of pink
Muscle, fibrin	Deep pinky red
Red blood cells	Orange red

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SAFETY:

Wear gloves, goggles and lab coat. Avoid contact and inhalation.
Always add the acid to the water.

SOLUTIONS

• Harris Hematoxylin

Product	CAS n°	Supplier & n°	Quantity
Hématoxyline solution Harris Gill II PAP1		Biosystems 3873.2500	

• 1% Acid-Alcohol

Product	CAS n°	Supplier & n°	Quantity
Absolute alcohol		VWR 20820.362	700 ml
Hydrochloric acid 37%	7647-01-0	Sigma 30721	10 ml
H2O MiliQ			290 ml

• Eosine – Phloxine working solution

1% Eosine Y stock solution

Product	CAS n°	Supplier & n°	Quantity
Eosine Y disodium salt	17372-87-1	Sigma E4382	10 g
H2O MiliQ			1000 ml

1% Phloxine B stock solution

Product	CAS n°	Supplier & n°	Quantity
Phloxine B	18472-87-2	Fluka 28550	1 g
H2O MiliQ			100 ml

Working solution (417ml)

Mix following solutions

- I. Eosine 50 ml,
- II. Phloxine 5 ml,
- III. Ethanol 95% 360 ml
- IV. Glacial acetic acid 2 ml.