Washington University School of Medicine
Neuromuscular Lab
CAP: 1923316
CLIA: 26D0652044
NY: PFI 3499

HEMATOXYLIN & EOSIN (H & E) STAIN PROTOCOL

PRINCIPLE:

This protocol is applied in the routine staining of cationic and anionic tissue components in tissue sections. This is the standard reference stain used in the study of histochemical tissue pathology.

SPECIMEN REQUIRED:

Snap frozen human striated muscle. (Use the 2-methylbutane freezing method)

METHOD:

Fixation: None. Use snap frozen tissue.

Technique: Cut 10 - 16 micron (12 µm) sections in cryostat from snap frozen biopsy. Attach **first and last** sections to a Superfrost Plus microscope slide.

Equipment:

Ceramic staining rack - Thomas Scientific #8542-E40
Columbia staining dish - Thomas Scientific #8542-C12
Columbia staining dish(jar) - Thomas Scientific #8542-E30
Forceps Latex gloves

Reagents:

Reagent alcohol - HPLC Fisher A995-4 or histological A962,
FLAMMABLE store at room temp. in a flammable cabinet
Eosin Y, disodium salt (Sigma #E-6003, store at room temperature)
Harris Hematoxylin Stain, acidified (Lerner Laboratories #1931382)(R.T.)
Permount - Fisher SP15-100, **FLAMMABLE; HEALTH HAZARD**
Xylenes (Fisher #HC700-1GAL, **FLAMMABLE**

Solutions:

I. Eosin Y, 1 % aqueous (store at room temperature)

Eosin Y dye	1 g
Deionized water	100 ml

2. Harris Hematoxylin, acidified (store at room temperature)
   Filter (Baxter #F2217-150, Grade 363, Qualitative) before use

3. Alcohol 50 %

reagent alcohol	~50 ml
deionized water	~50 ml

4. Alcohol 70 %

reagent alcohol	~70 ml
deionized water	~30 ml

5. Alcohol 80 %

reagent alcohol	~80 ml
deionized water	~20 ml

6. Alcohol 95 %

reagent alcohol	~95 ml
deionized water	~ 5 ml

Staining Procedure:

1. Place the slides with section in a metal staining rack.
2. Immerse sections in the filtered Harris Hematoxylin for 10 seconds.
3. Remove rack to a beaker with tap water.
4. Exchange tap water until the water is clear.
5. Immerse sections in EOSIN stain for ~30 seconds.
6. Remove rack to a beaker with tap water.
7. Exchange tap water until the water is clear.
8. Dehydrate in ascending alcohol solutions (50%,70%,80%,95% x 2, 100% x 2) in Columbia staining dish(jar)s - Thomas Scientific #8542-E30 .
9. Clear with xylene (3 - 4 x) in Columbia staining dish(jar)s - Thomas Scientific #8542-E30.
10. Mount coverslip onto the section on glass slide with Permount. or other suitable organic mounting medium.

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Results:

Nuclei and other basophilic structures are blue.
Cytoplasm and acidophilic structures are light to dark red.

REFERENCES:

1. Thompson, Samuel W. *SELECTED HISTOCHEMICAL AND HISTOPATHOLOGICAL METHODS,* Charles C. Thomas, Springfield, IL, 1966.
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2. Sheehan, D.C. and Hrapchak, B.B.: *THEORY AND PRACTICE OF HISTOTECHNOLOGY,* 2nd Edition; Battelle Memorial Institute, Columbus, OH, 1987.

12/26/2105