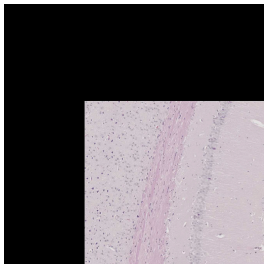
Sep 19, 2023 Version 2

Buck Institute Morphology H & E staining protocol V.2



DOI
dx.doi.org/10.17504/protocols.io.36wgq3n2ylk5/v2

Stella Breslin[1]

[1]Buck Institute for Research on Aging

Stella Breslin: Morphology Core standard protocol

Stella Breslin

DOI: https://dx.doi.org/10.17504/protocols.io.36wgq3n2ylk5/v2

Protocol Citation: Stella Breslin 2023. Buck Institute Morphology H & E staining protocol. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.36wgq3n2ylk5/v2**Version created by **Stella Breslin**

Protocol status: Working
We use this protocol and it's working

Created: September 19, 2023

Last Modified: September 19, 2023

Protocol Integer ID: 88039

Keywords: histological stain for ffpe slide, histological stain, red blood cell, staining protocol, ffpe slide, cytoplasm, connective tissue, tissue, blood, cell

Abstract

Histological Stain for FFPE slides
RESULTS: Nuclei-blue; Cytoplasm, red blood cells and connective tissue-shades of pink

Materials

1. Modified Mayes's Hematoxylin HXMMHPT StatLab Medical Products
2. 10% Acidic Acid
3. Scott's Tap Water (1L + 3.5g Sodium Bicarb + 20g Magnesium Sulfate pH 8.0) - Bluing Agent
4. Eosin Y Stain STE0150 StatLab Medical Products
5. reagents in 250 ml Coplin Jar

Troubleshooting

Safety warnings

Xylene (C_8H_{10})CAS No. 1330-20-7 is a colorless, flammable liquid with a sweet odor. Exposure to xylene can irritate the eyes, nose, skin, and throat. Xylene can also cause headaches, dizziness, confusion, loss of muscle coordination, and in high doses, death. Workers may be harmed from exposure to xylene.
from **https://www.cdc.gov/niosh/topics/xylene/**
Permount Mounting Media is toluene-based synthetic resin mounting medium. Use caution around uncured media.
https://www.cdc.gov/niosh/topics/toluene/

Before start

Slides can be placed in 60 C oven prior to deparaffinization.

Hematoxylin and Eosin Staining

1 Deparaffinization and rehydration: 2 x xylene 7 min, 100% ethanol 4 min, 95% 4 min, 80% 4 min, 70% 4 min, bring to water.

2 Hematoxylin staining for 30 seconds - 4 min. using filtered dye (usually 2 mins) - Modified Mayes's Hematoxylin HXMMHPT StatLab Medical Products

3 Rinse in ddH20 ×2

4 10% acetic acid rinse (acid into water) – 1 min

5 rinse in ddH20 ×2

6 TBS (1x) rinse or Scott's Tap Water (1L + 3.5g Sodium Bicarb + 20g Magnesium Sulfate pH 8.0) - 2 mins

7 Rinse in ddH20

8 Rinse in 70% ETOH - 1 min

9 Stain Eosin for 0.5 to 2 minutes (keep an eye on intensity of pink) – Eosin Y Stain STE0150 StatLab Medical Products

10 Rinse in 90% EtOH

11 Rinse in 2 × 95% EtOH, 2 × 100% EtOH, xylene

12 Coverslip with Permount Mounting Medium EMS #17986-01