

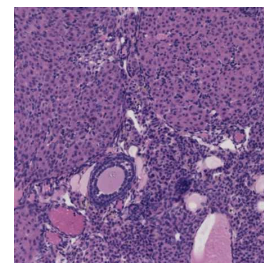
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Version 3

Buck Institute Morphology H & E staining protocol V.3

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Stella Breslin: Morphology Core standard protocol

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Protocol status: Working

We use this protocol and it's working

Created: September 28, 2023

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Keywords: FFPE, hematoxylin, eosin, H&E, stain, section, histology, slide, protocol histological stain for ffpe slide, staining protocol histological stain, red blood cell, ffpe slide, cytoplasm, connective tissue, tissue, 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/>

Permunt 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:
 1. 2 x xylene 7 min,
 2. 100% ethanol 4 min,
 3. 95% 4 min,
 4. 80% 4 min,
 5. 70% 4 min,
 6. 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 ddH₂O ×2
- 4 10% acetic acid rinse (acid into water) – 1 min
- 5 rinse in ddH₂O ×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 ddH₂O
- 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
 1. 2 × 95% EtOH,
 2. 2 × 100% EtOH,



3. xylene

12 Coverslip with Permount Mounting Medium EMS #17986-01