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Staining/Dehydration 10 micron Rat Heart

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DOI

<https://dx.doi.org/10.17504/protocols.io.webfbn>

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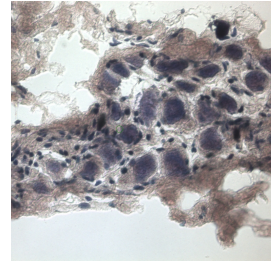
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Protocol status: Working

We use this protocol and it's working

Created: December 13, 2018

Last Modified: April 10, 2020

Protocol Integer ID: 18595

Keywords: dehydration 10 micron rat heart, micron rat heart, dehydration

Materials

MATERIALS

- ✕ EMD Millipore™ Ultrapure Water for Molecular Biology **Merck Millipore (EMD Millipore) Catalog #09-739-011**
- ✕ Cresyl Violet acetate pure high purity biological stain **Catalog #AC229630050**
- ✕ 1X Phosphate Buffered Saline Solution **Catalog #BP2438-4**
- ✕ Xylenes histological grade **Merck MilliporeSigma (Sigma-Aldrich) Catalog #534056-4L**
- ✕ Acetone for HPLC **Merck MilliporeSigma (Sigma-Aldrich) Catalog #650501-4L**
- ✕ Ethanol 200 Proof **Decon Labs Catalog #2716**
- ✕ Acetic Acid Glacial **Catalog #A38-212**

STEP MATERIALS

- ✕ 1X Phosphate Buffered Saline Solution **Catalog #BP2438-4**
- ✕ EMD Millipore™ Ultrapure Water for Molecular Biology **Merck Millipore (EMD Millipore) Catalog #09-739-011**
- ✕ 1X Phosphate Buffered Saline Solution **Catalog #BP2438-4**
- ✕ Ethanol 200 Proof **Decon Labs Catalog #2716**
- ✕ Ethanol 200 Proof **Decon Labs Catalog #2716**
- ✕ Xylenes histological grade **Merck MilliporeSigma (Sigma-Aldrich) Catalog #534056-4L**
- ✕ Xylenes histological grade **Merck MilliporeSigma (Sigma-Aldrich) Catalog #534056-4L**



Protocol materials

⊗ Acetic Acid Glacial **Catalog #A38-212**

⊗ Ethanol 200 Proof **Decon Labs Catalog #2716**

⊗ Xylenes histological grade **Merck MilliporeSigma (Sigma-Aldrich) Catalog #534056-4L**

⊗ Acetone for HPLC **Merck MilliporeSigma (Sigma-Aldrich) Catalog #650501-4L**

⊗ 1X Phosphate Buffered Saline Solution **Catalog #BP2438-4**

⊗ EMD Millipore™ Ultrapure Water for Molecular Biology **Merck Millipore (EMD Millipore) Catalog #09-739-011**

⊗ Cresyl Violet acetate pure high purity biological stain **Catalog #AC229630050**



Slide retrieval

- 1 Remove slide from -80 °C freezer. Keep slide covered and allow to come to room temperature for 00:01:00 or until frost on slide dissipates.

Fixation

- 2 Submerge slide into cold acetone (kept in -20 °C freezer) for 00:00:15

OCT removal

- 3 Gently dip slide 10 times into 1x PBS or until OCT appears to be washed off

1X Phosphate Buffered Saline Solution **Catalog #BP2438-4**

Staining

- 4 Submerge slide into 0.1% Cresyl Violet for 00:05:00

Rinse

- 5 Gently rinse slide by dipping into H₂O 10 times

EMD Millipore™ Ultrapure Water for Molecular Biology **Merck Millipore (EMD Millipore) Catalog #09-739-011**

Rinse



6 Gently dip slide 10 times into 1x PBS

 1X Phosphate Buffered Saline Solution **Catalog #BP2438-4**

Dehydration

7 Dip and agitate slide 2 times into 95% EtOH

8 Dip and agitate slide 2 times into 100% EtOH

 Ethanol 200 Proof **Decon Labs Catalog #2716**

9 Submerge slide into 100% EtOH for  00:00:15

 Ethanol 200 Proof **Decon Labs Catalog #2716**

Slide retrieval

10 Gently dip slide 2 times into Xylene

 Xylenes histological grade **Merck MilliporeSigma (Sigma-Aldrich) Catalog #534056-4L**

Safety information

Flammable liquids (Category 3), H226
Acute toxicity, Inhalation (Category 4), H332
Skin irritation (Category 2), H315
Eye irritation (Category 2A), H319
Carcinogenicity (Category 2), H351



11 Submerge slide into Xylene for  00:00:20



Xylenes histological grade **Merck MilliporeSigma (Sigma-Aldrich) Catalog #534056-4L**

Safety information

Flammable liquids (Category 3), H226
Acute toxicity, Inhalation (Category 4), H332
Skin irritation (Category 2), H315
Eye irritation (Category 2A), H319
Carcinogenicity (Category 2), H351

12 Allow slide to dry under chemical fume hood for  00:05:00
Proceed to LCM/LMD