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

Created: Dec 13, 2018

Last Modified: Apr 10, 2020

PROTOCOL integer ID:
 18595

Staining/Dehydration 10 micron Rat Heart

In 2 collections

Shaina Robbins¹, Sean Nieves¹

¹Thomas Jefferson University

SPARC

Tech. support email: info@neuinfo.org

Shaina Robbins

Thomas Jefferson University

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 **Contributed by users Catalog #AC229630050**

✕ 1X Phosphate Buffered Saline Solution **Contributed by users Catalog #BP2438-4**

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

✕ Acetone for HPLC **Sigma – Aldrich Catalog #650501-4L**

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

✕ Acetic Acid Glacial **Contributed by users Catalog #A38-212**

STEP MATERIALS

✕ 1X Phosphate Buffered Saline Solution **Contributed by users Catalog #BP2438-4**

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

✕ 1X Phosphate Buffered Saline Solution **Contributed by users Catalog #BP2438-4**

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

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

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

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 **Contributed by users 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**

- 6 Gently dip slide 10 times into 1x PBS

⊗ 1X Phosphate Buffered Saline Solution **Contributed by users 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 #534056-4**

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 #534056-4**

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