

1. Paraffin sections were placed at 60°C overnight to melt paraffin.
2. Paraffin sections were then placed in xylene (Xylenes Reagent Grade ACS Histology/Lab Grade Cat # 399000000 Pharma-AAPER) 3X for 10 minutes each.
3. Rinsed slides in a series of 100% ETOH washes. 20 dips for the first wash, 3 minutes for the second wash, and 20 dips for the third wash.
4. Rinsed slides in a series of 95% ETOH washes. 20 dips for the first wash, 3 minutes for the second wash, and 20 dips for the third wash.
5. Rinsed slides in 70% ETOH for 3 min.
6. Rinsed slides for 3 minutes in running dH₂O.
7. Slides were then stained in filtered Hematoxylin for 60 seconds. (Gold Standard Harris Hematoxylin, Acidified, Mercury; Cat # 24245 Polysciences Inc.).
8. Rinsed slides in tap water until water turned cleared then rinsed in dH₂O
9. Rinsed slides in Lithium Carbonate: 20 dips. (Lithium Carbonate ACS Grade; Cat # 255823-500G Sigma Aldrich). Lithium carbonate is prepared by over-saturating in dH₂O.
10. Rinsed slides in dH₂O 3X 20 dips.
11. Rinsed slides in 70% ETOH for 20 dips.
12. Rinsed slides in 80% ETOH for 20 dips.
13. Stained slides in Eosin for 15 seconds. (Eosin Y 0.5% Alcohol Solution, Cat # 09859 Polysciences Inc.).
14. Rinsed slides in a series of 95% ETOH washes. 10 dips for the first and second washes, 15 dips in the third and fourth washes.
15. Rinsed slides 3X in 100% ETOH. 10 dips for the first and second washes and 15 dips in the third and fourth washes.
16. Rinsed slides in 2X in Xylene, followed by 6 more washes in xylene.
17. Coverslip with Permount. (Permount Toulene Solution UN 1294, Cat # SP-15-500 Fisher Scientific).

Tissue Used:

LMH-15-D024-RLL-9C2.10

Age: 4 Months Old