

1. For Frozen tissue, rinsed slides for 2X in PBS, then Incubated slides for 5 min in 4% PFA/PBS, then rinsed slides 1X in PBS.
2. Rinsed slides for 3 minutes in running dH₂O.
3. Slides were then stained in filtered Hematoxylin for 60 seconds. (Gold Standard Harris Hematoxylin, Acidified, Mercury; Cat # 24245 Polysciences Inc.).
4. Rinsed slides in tap water until water turned cleared then rinsed in dH₂O
5. 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.
6. Rinsed slides in dH₂O 3X 20 dips.
7. Rinsed slides in 70% ETOH for 20 dips.
8. Rinsed slides in 80% ETOH for 20 dips.
9. Stained slides in Eosin for 15 seconds. (Eosin Y 0.5% Alcohol Solution, Cat # 09859 Polysciences Inc.).
10. 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.
11. Rinsed slides 3X in 100% ETOH. 10 dips for the first and second washes and 15 dips in the third and fourth washes.
12. Rinsed slides in 2X in Xylene, followed by 6 more washes in xylene.
13. Coverslip with Permout. (Permout Toulene Solution UN 1294, Cat # SP-15-500 Fisher Scientific).

Tissue Used: LMH-D008-LLL-8B4.18

Gender: Female

Age: 20 Months