

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 incubated in 3% Glacial Acetic Acid ([S101](#), Poly Scientific) for 3 minutes.
8. Slides were then incubated in filtered Alcian Blue ([S111A](#), Poly Scientific) for 30 minutes.
9. Wash slides under cold running tap water for 10 minutes.
10. Incubate for 2 minutes in Nuclear Fast Red ([S248](#), Poly Scientific).
11. Wash under flowing dH₂O until excess Nuclear Fast Red is washed away.
12. Incubated slides in 90% ETOH for 20 dips, repeat in different 90% ETOH.
13. Incubated slides in 100% ETOH for 20 dips, repeat in different 100% ETOH.
14. Washed slides in 2X in Xylene, followed by 6 more washes in xylene.
15. Coverslip with Permount. (Permount Toulene Solution UN 1294, Cat # [SP-15-500](#) Fisher Scientific).

Tissue Used:

LMH-D044-RLL-9A2.12

Gender: Male

Age: 6 Days-Old