Carmine Alum-stained Whole mount preparation

• Fix the tissue for 2 hours in 4% paraformaldehyde at 4°C (see 4% PFA protocol).

• Rinse the tissue in PBS and stain in carmine alum solution overnight at room temperature.

FOR TISSUE TO BE USED FOR IMMUNOSTAINING:
• Rinse cassettes with 70% ethanol and store tissues in 70% ethanol until ready to be embedded in paraffin. The glands can be flattened between glass slides to capture whole mount images before being processed.

FOR PERMANENT WHOLE-MOUNTING:
• Wash cassettes in 70% ethanol for 1 hour
• Wash cassettes in 95% ethanol for 1 hour
• Wash cassettes in 100% ethanol for 1 hour
• Clear tissue in xylene overnight
• Store tissue in methyl salicylate.

Carmine Alum staining solution:
• Carmine, Sigma C-1022
• Aluminum Potassium Sulfate, Sigma A-7167

1 g carmine
2.5 g aluminum potassium sulfate
500 ml distilled water

• Boil for 20 minutes
• Adjust final volume to 500 ml with water
• Filter sterilize and add a crystal of thymol as a preservative.
• Store at 4°C.

Note: The staining solution can be reused.

Representative images: