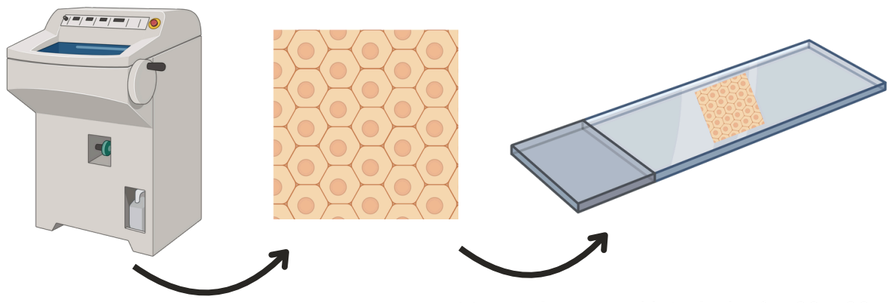
Protocol
CHROMOGENIC IMMUNOHISTOCHEMISTRY STAINING OF FROZEN TISSUE

1. Tissue Sectioning & Fixation

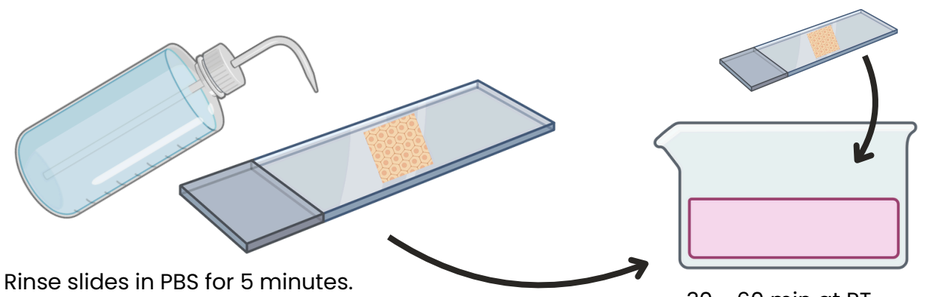


Cut 5–10 µm thick tissue sections using a cryostat at -20°C

Mount tissue and let air dry for 20 - 30 Minutes

Fix sections:
Option A (acetone): Fix in cold acetone (-20°C) for 10 minutes.
Option B (PFA): Fix in 4% PFA for 10 minutes, then rinse in PBS.
Air dry the slides again for 10 minutes.

2. Permeabilization & Blocking

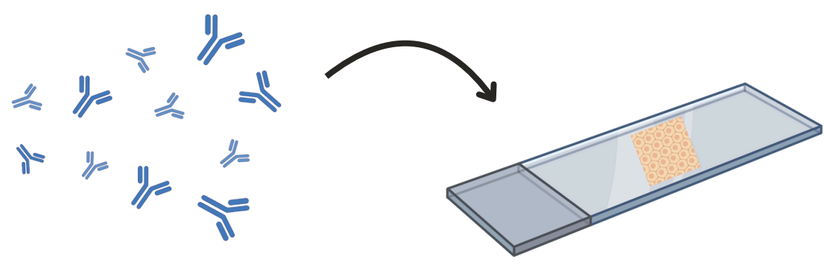


Rinse slides in PBS for 5 minutes.

30 - 60 min at RT

Rinse slides in PBS for 5 minutes.
(Optional) Incubate with 0.1–0.3% Triton X-100 in PBS for 10 minutes if intracellular targets are being stained.
Block with 5–10% normal serum (same species as the secondary antibody) or 1–5% BSA in PBS for 30–60 minutes at room temperature.

3. Primary Antibody Incubation

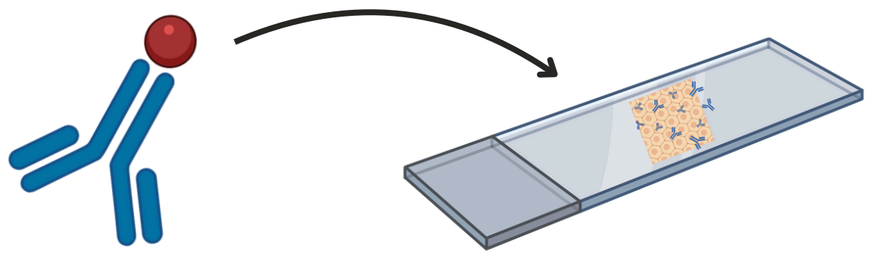


Diluted primary antibody

4°C Overnight in humidified chamber

Dilute the primary antibody as recommended by the manufacturer.
Apply to each section and incubate:
1–2 hours at room temperature or
Overnight at 4°C in a humidified chamber.
Rinse in PBS, 3 × 5 minutes.

4. Secondary Antibody & Detection

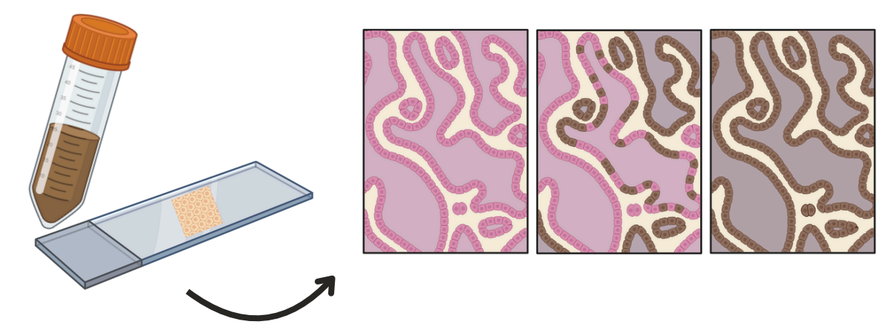


HRP- or AP-conjugated secondary antibody

30 - 60 min at RT

Incubate sections with the enzyme-conjugated secondary antibody for 30–60 minutes at room temperature.
Rinse in PBS or TBS, 3 × 5 minutes.

5. Chromogenic Substrate Development

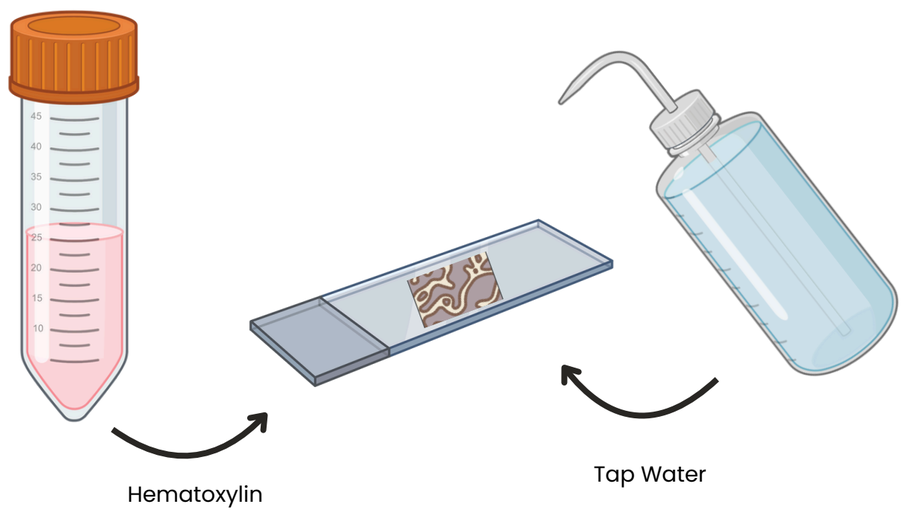


DAB (brown), AEC (red), or other chromogens

Prepare the chromogen fresh just before use.
Apply substrate to sections and monitor color development under a microscope (typically 5–15 minutes).
Stop the reaction by rinsing in distilled water.

⚠️ **Note: AEC is alcohol-soluble; avoid alcohol-based mounting.**

6. Counterstaining & Mounting



Hematoxylin

Tap Water

Counterstain with hematoxylin for 30–60 seconds.
Rinse in tap water and blueing reagent if required.

Mount:
For DAB: Dehydrate, clear in xylene, and mount with a permanent medium.
For AEC: Mount with aqueous mounting medium (do not dehydrate).

Images created with https://BioRender.com

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