Dec 04, 2025

Immunohistochemistry _ Chromogenic_FFPE

DOI
https://dx.doi.org/10.17504/protocols.io.kxygx442dl8j/v1

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Protocol Citation: Yue Ma 2025. Immunohistochemistry _ Chromogenic_FFPE. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx442dl8j/v1

Protocol status: Working
We use this protocol and it's working

Created: October 25, 2025

Last Modified: December 04, 2025

Protocol Integer ID: 230756

Keywords: ASAPCRN, ffpe sample dab, good for ffpe sample dab, ffpe

Abstract

The protocol is good for FFPE sample DAB staining

Guidelines

4. Wash sections with PBST or TBST (0.1% Tween20) for 10 mins, repeat for 3 times.

5. **Quenching**: incubate with 0.3% H2O2 (stock 30%, 4°C, SigmaH1009) in methanol for 10mins at 4°C.

6. Wash sections with PBS or TBS for 5 mins, repeat for 3 times.

7. Secondary antibody incubation: dilute biotinylated goat anti-xx IgG (rat 1:400, rabbit 1:500, mouse 1:500) in TBST, incubate for 2h at RT or 24h at 4°C.

8. Wash sections with PBST or TBST (0.1% Tween20) for 10 mins, repeat for 3 times.

9. Prepare 30 mins before use ABC (avidin-biotin complex) mix: 10 ml PBS+ 4 drops of reagent A(vortex)+ 4 drops of reagent B(vortex), let stand at RT for 30 mins.

10. ABC incubation for 1h at RT.

11. Wash sections with PBST or TBST (0.1% Tween20) for 10 mins, repeat for 3 times. Then wash with PBS for 5 mins, repeat for 2 times.

12. Prepare DAB solution: 10 ml PBS+ 4 drops of reagent 1 (buffer PH7.5, vortex) + 8 drops of reagent 2 (DAB, vortex) + 4 drops of reagent 3 (H2O2, vortex). Revelation with DAB chromogen until grey staining is available.

13. Counter staining (optional):

- 0.1% Cresyl violet/Nissl staining: 15 mins.
- Hematoxylin staining: drop slides in Harris Hematoxylin Solution (Sigma) for 5 mins, then transfer slides to tap water for 5 mins, then 0.5% hydrochloric acid in 70% ethanol (start from 30s, keep until staining look good), then transfer slides to tap water for 5 mins.

14. Dehydrate: add slides to 70%, 95%, 100% ethanol for 5 mins each.

15. Clearance: 1min in Xylene I, then in Xylene II (use fresh solution) until mounted.

16. Coverslip mounting with Entellan (Merk) medium.

Bake slides

1 Bake slides in a dry oven for 1h at 60°C.

Deparaffinize and hydrate tissue sections

2 Incubate the slides in xylene in the fume hood for 5 min.

3 Place in the second xylene in the fume hood for 5 min.

4 Remove the slides from the xylene, and immerse them in 100% ethanol for 2 min.

5 Place in the second 100% ethanol for 2 min.

6 Immerse the slides in 90%, 80%, 70% EtOH, each for 2 min, and then distilled water for 1 min, then keep the slides in PBS until ready to perform the Antigen Retrieval. Do not allow the slides to dry out.

Antigen retrieval

7 Prepare antigen retrieval buffer base on the antibody's data sheet. [Tris/EDTA buffer pH 9.0; 10x citrate buffer 0.01M, pH6; 10x HIER pH 6 (Abcam)]

8 Transfer the slides into the container with antigen retrieval buffer.

9 Place the container into the steamer.

10 Turn on the steamer, heat the antigen retrieval buffer to 95-100°C, start counting for 30 min.

11 Remove the container from the steamer and allow it to cool down for 20 min (~60°C).

Staining

12 Draw a circle around the tissue with Hydrophobic Barrier PAP Pen (Vector), let it dry.

13 Blocking: 10% normal goat serum + 0.1% Triton X in PBS or TBS for 1h at RT.

14 Primary antibody incubation: dilute antibody in 5% normal goat serum+ 0.1% Triton X in PBS or TBS for 24-48h at 4°C.

15 Wash sections with PBST or TBST (0.1% Tween20) for 10 mins, repeat for 3 times.

16 **Quenching**: incubate with 0.3% H*2O*2 (stock 30%, 4°C, SigmaH1009) in methanol for 10mins at 4°C.

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18 Secondary antibody incubation: dilute biotinylated goat anti-xx IgG (rat 1:400, rabbit 1:500, mouse 1:500) in TBST, incubate for 2h at RT or 24h at 4°C.

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