
Immunoperoxidase Staining Protocol

1. Dewax slides as follows:

Xylene	5 mins
Xylene	5 mins
100% Ethanol	2 mins
100% Ethanol	2 mins
95% Ethanol	2 mins
2. Wash 3x with dH₂O for 2 minutes each.
3. Steam in pressure cooker with 200mL 1x Citric acid for 15 minutes.
4. Let the slides cool in pressure cooker for 10 minutes before releasing pressure.
5. Release pressure and move slides to hot dH₂O for 2 minutes.
6. Flush with running tap water for 5 minutes.
7. Put slides in hydrogen peroxide (3% in dH₂O) for 10 minutes.
8. Rinse in dH₂O by dipping a few times
9. Rinse in wash buffer for 5 minutes.
10. Dry slides around tissue sample and draw a ring with hydrophobic pen.
11. Block with normal serum (3% goat serum, sometimes other serum depending on reagents and stain) for 10 minutes. (20 minutes for frozen)
12. Add 150uL to 200uL of primary antibody directly on slide, can dilute antibody using antibody dilutant. Incubate for 1 hour at room temperature.
13. Wash 3x in wash buffer for 5 minutes each.
14. Add 150uL to 200uL of secondary antibody directly on slide. Time varies according to specific experiment.
15. Wash 3x in wash buffer for 5 minutes each.
16. Make DAB according to instructions in box and add directly on slide. Let it stain for 10 minutes.
17. Wash 3x in wash buffer for 5 minutes each.
18. Rinse with tap water.
19. Dip in Hematoxylin for 30seconds to 1 minute max)
20. Rinse in tap water for approximately 2 minutes, until tissues samples look blue.
21. Dehydrate tissue sample as follows:

95% Ethanol	2 mins
95% Ethanol	2 mins
100% Ethanol	2 mins
100% Ethanol	2 mins
Xylene	5 mins
Xylene	5 mins
22. Drop 3 drops of mounting media directly on slide and carefully place coverslip

A few general points:

1. You will need to titrate the primary antibody. We generally recommend starting at 1 ug/mL for IHC, but of course, you should consult the TDS for the primary. As an example, Abcam will provide working concentrations or dilutions for their primary Abs. 30-60 minute incubation time, but 30 works just fine.
2. Our secondary antibody is RTU, so no need for working concentration. Generally, we recommend 30-60 minutes incubation time for the Secondary HRP. 30 minutes works just fine.
3. Citric Acid pH6.0 heat induced epitope retrieval (HIER) buffer is a very standard HIER buffer. I can provide a very basic recipe for this if desired.