FREE-FLOATING IMMUNOHISTOCHEMISTRY

This procedure may be used for tissue sections mounted on slides and for free-floating frozen tissue sections (usually 30-50 microns) collected and processed in 24-well culture plates. The procedure is compatible with free-floating frozen and vibratome tissue sections fixed with 4% paraformaldehyde or Bouin's fix. It can also be used for sections already mounted on glass slides (paraffin tissue sections fixed with Carnoy's, Bouin's, 10% formalin and 4% paraformaldehyde) that will be processed in coplin jars.

1. Rinse tissue sections for 5 min in PBS.
2. Make a 50 ml aliquot of 0.03% H_2O_2 (take 250 μl of 30% H_2O_2 and dilute it in 25 ml PBS).
3. Incubate the tissue sections for 10 min in H_2O_2 at room temp to quench endogenous peroxidases.
4. Rinse the tissue for 2 X 5 min in PBS.
5. Block the tissue in 10% NGS/0.1% Triton-X 100 all in PBS for 2 hrs at RT.
6. Incubate the tissue in primary antibody O/N at room temp or 4 degrees as required by the specific antibody (diluted in a new aliquot of the blocking solution).
7. Rinse the tissue for 3 X 5 min in PBS.
8. Incubate the tissue in secondary antibody for 2 hrs at room temp. I use the DAKO secondary antibodies Goat anti-rabbit conjugated to HRP and goat ant-mouse conjugated to HRP both diluted 1:200 (diluted in a new aliquot of the blocking solution).
9. Rinse the tissue for 3 X 5 min in PBS.
10. Make your DAB solution as follows (always made fresh):
    Take 1 X 10 mg tablet and dissolve in 40 ml PBS
    Vortex vigorously to dissolve
    Add 10 microlitres of 30% H2O2
    Vortex for 10 sec to mix
11. Pass the DAB through a syringe fitted with a filter unit to remove particles of DAB that did not dissolve.
12. Apply the DAB solution to your tissue sections.
13. The DAB reaction produces a brown reaction product and the reaction tends to proceed very quickly with most antibodies.
14. If the reaction takes longer than a couple of minutes to develop, incubate the tissue in the dark to avoid light induced precipitation of the DAB product (precipitated reaction product will stick to the surface your tissue and will also give high background staining).
15. Stop the reaction at the desired color intensity by washing the tissue in PBS.
16. Dehydrate the tissue in an ethanol series and clear with Xylene or Histoclear.
17. Coverslip using permount or DPX.

DAB needs to be inactivated before it is thrown out. Add DAB inactivating solution (3% potassium permangenate, 2% sodium carbonate) to your DAB (1:1) and then discard the neutralized DAB using hazardous waste procedures. DAB is carcinogenic so pay special attention to any instruments and containers that touch the DAB.

This procedure can also be used for immunofluorescence staining. You can omit the quenching of endogenous peroxidases for fluorescent immunohistochemistry. In addition, you will need to mount the tissue using an aqueous mounting media that will preserve your fluorescent tags.

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