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Immunocytochemistry Protocol

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Equipment:

Frozen Tissue
Microtome
Gelatin coated glass slides
Coplin Jars

Reagents:

DPBS with 5% FCS and 0.05% Tween-20
Tris buffer
Antibodies
Sodium bicarbonate buffer
Diaminobenzadine (DAB) workign solution
Osmium Tetroxide (OsO_4) working solution
1% Gluteraldehyde in DPBS
95% ETOH
100% ETOH
Xylene (or Histoclear)
Permount

Method:

1) Cut 6 μm sections of tissue; mount on gelatin coated slides.
2) Immerse 1-2 seconds in cold acetone (on ice).
3) Air dry slide 30-60 seconds.
4) Immerse 15-60 minutes in DPBS + 5% FCS + 0.05% Tween-20.
5) Wash 5 minutes in Tris (0.05 M, pH 7.6).
6) Stain 45-60 minutes with 50-100 μl primary antibody (diluted in Tris).
7) Wash 2 minutes in Tris.
8) Wash 3 minutes in Tris.
9) Stain 45-60 minutes with 50-100 μl secondary antibody (diluted in Tris).
10) Wash 2 minutes in Tris.
11) Wash 3 minutes in Sodium Bicarbonate buffer (0.1M, pH 8.20).
12) Stain 45-60 minutes with HRP-Avidin (diluted 1:5000 in Sodium Bicarbonate buffer).
13) Wash 2 minutes in Sodium Bicarbonate buffer.
14) Wash 3 minutes in Tris.
15) Develop for 10 minutes in DAB solution in Tris buffer.
16) Wash 2 minutes in Tris.
17) Wash 3 minutes in DPBS.
18) Enhance staining with 100 μl of 1% OsO_4 in DPBS for 1-5 minutes.
19) Wash 2 minutes in DPBS.
20) Post-fix slides 5 minutes in 1% Gluteraldehyde in DPBS.
21) Dehydrate 5 minutes in 95% alcohol.
22) Dehydrate 5 minutes in 100% alcohol.
23) Dehydrate 5 minutes in Xylene or Histoclear.
24) Coverslip slides using Permount.