Immunocytochemistry with Mouse Monoclonal Antibodies

This procedure is for free floating sections in trays that have bins which hold a few ml each. Make sure sections are careful transferred with paintbrushes. Limit the # sections per bin so they do not overlap and stay immersed.

Day 1

Wash with 0.1M Tris buffer (see Stock Solutions) 3 x 5 min
Incubate in 1% H_20_2 for 1-2 min
    1 ml 30% H202 and 29 ml 0.1M Tris buffer. Always make fresh.
    If bubbles form, remove the sections and put them immediately into 0.1M Tris buffer
Wash in 0.1M Tris buffer 3 x 5 min
Wash in Tris A, 10 min
Wash in Tris B, 10 min
Incubate in 10% Normal Horse Serum (Vector Laboratories) for 30 min
    4 ml Normal Horse Serum and 36 ml Tris B
Wash in Tris A, 10 min
Wash in Tris B, 10 min
Add primary antibody diluted in Tris B
Incubate overnight at room temperature on a rotating shaker

Day 2

Wash in Tris A, 10 min
Wash in Tris B, 10 min
Incubate with Anti-mouse IgG in Horse (secondary antibody; Vector) 1:400 for 45 min
    100 µl IgG in 40 ml Tris B
Wash in Tris A, 10 min
Incubate in Tris ABC 1:1000 for 2 hrs
    40 µl Reagent A
    40 µl Reagent B
    40 µl Tris B
Wash in 0.1M Tris buffer 3 x 5 min
Incubate in diaminobenzidine (DAB; Sigma)
    1 DAB tablet (10 mg)
    20 ml 0.1M Tris buffer
    40 ul Ammonium chloride stock (2g/10ml)
    20 ul Glucose oxidase stock (0.0075g/2.5 ml)
    160 ul D+-glucose stock (2.5g/10ml)
Wash in 0.1M Tris buffer, 3 x 5 min
Mount on treated slides, allow to dry overnight
Dehydrate in ethanol sequentially:
    70% 10 min; 90% 10 min; 95% 10 min; 100% 10 min; 100% 10 min
Clear in Xylene, 10 min
Coverslip in Permount (Fisher)

<u>For Nickel intensification</u>, add $NiCl_2$ to the DAB solution as follows
    5 mg per 20 ml

<u>For NovaRed</u> (Vector) instead of DAB:
    5 ml dH_20
    3 drops Reagent 1
    2 drops Reagent 2
    2 drops Reagent 3
    2 drops H_20_2 solution
    wash in dH_20 afterwards, 2 x 5 min, followed by 0.1M Tris buffer, 2 x 5 min