Immunocytochemistry with Rabbit Polyclonal Antibodies

These procedures are for free-floating sections using trays that have bins holding approx. 5 ml. Do not fill the bins with too many sections or else staining will be uneven. Transfer with paintbrushes. All solutions are described in the Stock Solutions pdf.

Day 1

Wash with 0.1M Tris buffer, 3 x 5min
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, 3x5 min
Wash in Tris A, 10 min
Wash in Tris B, 10 min
Incubate in 10% Normal Goat Serum (S-1000, Vector Laboratories) for 30 min
    4 ml Normal Goat 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

Day 2

Wash in Tris A, 10 min
Wash in Tris B, 10 min
Incubate with Anti-Rabbit IgG in Goat (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 Tris 3x5 min
Incubate in diaminobenzidine (DAB; Sigma)
    1 DAB tablet (10 mg)
    20 ml 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, 3x5 min
Mount on treated slides, allow to dry overnight
Dehydrate in ethanol
    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 (Vector)</u> stain 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