Immunohistochemistry

I. Immunohistochemistry using the avidin-biotin complex (ABC) method

1. Brain sections are treated with methanolic hydrogen peroxide [1-3% H_2O_2 (Fisher Scientific, H325-500) in Tris-buffered saline (TBS) containing 10% methanol] for 30 min to block nonspecific endogenous peroxidase activity.
2. The sections are then rinsed (3 x 10 min) in a diluting buffer [TBS containing 0.4% Triton X-100, 1% normal goat or rabbit serum (Rockland Immunochemicals for Research, D204-00 and D209-00, respectively) and 2% bovine serum albumin (BSA, Fisher Scientific, BP1605-100), pH 7.4] and blocked (30min at RT) with 20% normal serum from the species donating the secondary antibody (e.g., goat or rabbit) to reduce non-specific background staining.
3. Sections are then incubated in appropriate concentrations of primary antibodies diluted in the diluting buffer overnight at 4°C.
4. After washing with the diluting buffer for 10 min 3 times, the tissue is incubated first in a biotinylated-secondary antibody (1:200 dilution, Vector Laboratories, BA-1000 for anti-rabbit, BA-2000 for anti-mouse) for 30 min at RT, rinsed, and subsequently in preformed ABC according to the manufacturer's instruction (Vector Standard ABC, Vector Laboratories, PK-4000) for 1 h at RT.
5. The final reaction is achieved by treating the sections with hydrogen peroxide and 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Vector Substrate Kit, Vector Laboratories, SK-4100) for 5 to 10 min.

6. Free-floating brain sections are mounted on gelatin coated slides, to help with tissue adhesion. Sections are then dehydrated in an ascending ethanol series, cleared with xylene and coverslipped with Permount.

Negative controls consist of either incubating tissue in nonimmune sera or omitting incubation in primary antisera.

II. Immunohistochemistry using immunofluorescent labeling

1. Brain sections (either vibratome, frozen or paraffin sections) are rinsed in the diluting buffer, blocked and then incubated in primary antisera overnight at 4°C. For double labeling studies, antibody cocktails can be made from two primary antisera from different host species.
2. After washing with the diluting buffer for 10 min 3 times, sections are incubated in Alexa-conjugated secondary antibodies of the appropriate species (Invitrogen, e.g., A11034, A11036, A11029, A11031) for 4 h at RT (dilutions of secondary antibodies range from 1:500 to 1:1,000).
3. After washing with TBS, the sections are mounted on gelatin-coated slides and coverslipped in an aqueous anti-fade mounting medium such as Gelmount (Biomeda, M01) or VectaMount AQ (Vector Laboratories, H-5501).
4. Sections are visualized by epifluorescent or confocal microscopy.