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Immunohistochemistry of Paraffin Sections

Sacrifice and tissue processing

Mice are deeply anesthetized with pentobarbital sodium (Pental). Brain is fixed by transcardial perfusion, first with 20 ml of phosphate buffered saline (0.02M PBS, pH 7.4), then with 80-100 ml of ice-cold 4% paraformaldehyde in 0.1M PBS, pH 7.5 containing sucrose 4%. Brain is cut in coronal blocks and further fixed by immersion in the same fixative, refrigerated, overnight. Other organs, heart or kidney, are immersed whole in the fixative. Tissues are processed for paraffin sectioning by standard methods. Paraffin sections are mounted on glass slides and kept refrigerated until immunohistochemical processing.

Staining procedure

1. Slides are deparaffinized in xylene 10 min. Then slides are rehydrated in descending concentrations of alcohol and from 70% alcohol solution are transferred to 0.02M PBS and refrigerated until initiation of endogenous peroxidase quench.
2. Endogenous peroxidase activity is quenched by incubation with 0.2 % hydrogen peroxide in 0.1M phosphate buffer pH 7.3 containing 0.2% Triton X-100 for 25 minutes at room temperature.
3. Sections are rinsed in 0.02M PBS.
4. Sections are treated with trypsin: Trypsin, Sigma type II, 0.1% in 0.1M Tris HCl buffer, pH 7.6 at room temperature for 20 min. After two rinses in 0.02M PBS, sections are incubated with the primary antibody.
5. Sections may be treated with sodium dodecyl sulfate 0.3% at room temperature for a period ranging from 5-30 minutes. It is recommended that the end user try a range of intervals.
6. Sections are incubated with the primary antiserum in a medium containing 0.001% Trypsin inhibitor (Sigma soybean type IV), 0.3% Triton X-100, 0.05% Tween 20, 4% normal donkey serum (NDS), for 1 hour at room temperature and then overnight refrigerated.
7. Sections are rinsed in 0.02M PBS, containing 4% NDS.
8. At this point, staining may proceed with various types of secondary antibodies.
   Two **alternative** procedures are described here:
   Protocol A: Sections are incubated with biotinylated donkey anti-rabbit (from Chemicon USA, catalog number AP182B) diluted 1:400 in 0.02M PBS, containing 0.3% Triton X-100, 0.05% Tween 20, and 4% NDS, for 1 hour at room temperature and then overnight refrigerated.
7. Sections are rinsed in 0.02M PBS containing 4% NDS, 2x 5 min.
8. Sections are incubated with extravidin-peroxidase (Sigma Catalog number E2886) diluted 1:100 in 0.02M PBS, for 45 minutes at room temperature.
9. Sections are rinsed in 0.02M PBS, 3x 5 minutes.

   Protocol B: Sections are incubated with horseradish peroxidase labeled donkey anti-rabbit (from Chemicon USA, catalog number AP182P), 1:400 in 0.02M PBS, containing 0.3% Triton X-100, 0.05% Tween 20, and 4% NDS for 1 h at room temperature and then refrigerated overnight.
7. Sections are rinsed in 0.02M PBS containing 4% NDS, 2x 5 min.
8. Sections are rinsed in 0.02M PBS, 2x 5 minutes.

Color development

1. Sections are incubated with a solution of diaminobenzidine (Sigma catalog number D5637) at the concentration of 0.0125% and containing 0.05% nickel ammonium sulfate for 10 minutes at room temperature.
2. Sections are transferred to the same DAB solution but with added hydrogen peroxide at a final concentration of 0.0015%. Duration of incubation should be adjusted by the end user.
3. Sections are rinsed in 0.02M PBS, 4x 10 minutes.
4. Sections are mounted on glass slides (gelatinized or coated by any other type of adhesive material) and allowed to dry.
5. Sections are dehydrated in ascending series of ethanol concentrations (70%, 90%, 100%, 5 minutes in each), delipidated in xylene (10 minutes) and coverslipped in Permount (or any other xylene diluted adhesive).