alomone labs

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Protocol for Immunohistochemistry of Kidney Sections

Sacrifice and tissue processing

Rats are deeply anesthetized with pentobarbital sodium (Pental). Kidney is fixed by transcardial perfusion, first with 50 ml of phosphate buffered saline (0.02M PBS, pH 7.4) containing heparin (5U/ml), then with 220 ml of ice-cold 4% paraformaldehyde in 0.1M PBS, pH 7.5 containing sucrose 4%. Kidney blocks are embedded in paraffin, cut at 7 µm intervals and sections are mounted on SuperFrost Plus slides.

Phosphate buffered saline solutions:	
0.02M PBS	
Dibasic phosphate 0.2M	80 ml
Monobasic phosphate 0.2M	16 ml
Distilled deionized water	860 ml
NaCl	8 grams
0.1M PBS	
Dibasic phosphate 0.2M	220 ml
Monobasic phosphate 0.2M	50 ml
Distilled deionized water	270 ml
NaCl	2.45 grams

Staining procedure

1. Sections are deparaffinized: 10 minutes in Xylene, 10 min. in 100% Ethanol, 6 min. in 90% Ethanol, 3 min. in 70% Ethanol, 5 min. in 0.02M PBS.
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, 2x 5 minutes.
4. Sections are incubated with the primary antiserum in a medium containing 0.3% Triton X-100, 0.05% Tween 20, 4% normal donkey serum (NDS), for 1 hour at room temperature and then overnight refrigerated.
5. Sections are rinsed in 0.02M PBS, containing 4% NDS, 2x 5 minutes.
6. Biotinylated secondary antibody: 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.

Color development

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.

1. 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.
2. Sections are rinsed in 0.02M PBS, 4x 10 minutes.
3. 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).