Immunohistochemical staining

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Abstract

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Protocol

Step 1.

Slides are deparaffinized by immersion in xylene 2 times for 2 hr.

Step 2.

Slides are rehydrated by immersion in 100% ethanol (10 min.), in 95% ethanol (10 min), in 85% ethanol (10min). Slides are washed with PBS (3 times, 3 min).

Step 3.

Antigen retrieval is performed by heating the slides in 10 mM citrate buffer (pH 6.0) at 98°C for 10 min in a microwave oven. Slides are washed with PBS (3 times, 3 min).

Step 4.

The endogenous peroxidase activity was blocked by incubation in 3% H2O2 for 20 min. Slides are washed with PBS (3 times, 3 min).

Step 5.

Antibody incubations were performed in phosphate-buffered saline (PBS) supplemented with 10% goat serum for 20 min at room temperature.

Step 6.

Slides are incubated 16hr at 4°C in a humidifying box with primary antibody. Negative controls are made with PBS alone.

Step 7.

Slides are washed with PBS (3 times, 3 min).

Step 8.

Slides are incubated with biotinylated secondary antibody for 30 min in a humidifying box at room temperature.

Step 9.

Slides are washed with PBS (3 times, 3 min).

Step 10.

Slides are incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Bio-technology) for 20 min in a humidifying box at room temperature.

Step 11.

Slides are washed with PBS (3 times, 3 min).

Step 12.

Signals were visualized by using 3 ’3Pdiaminobenzidine (DAB; Sigma, UK) for 1 minute and terminated by incubated in distilled water.

Step 13.

Slides are washed with distilled water and counterstained with hematoxylin for 10 seconds.

Step 14.

Slides are washed with running water for 6 min.

Step 15.

Slides are dehydrated by immersion in 70% ethanol (1 min), in 95% ethanol (5 min), in 100% ethanol (2 times, 5 min) and in xylene (2 times, 20 min).

Step 16.

Cover slides are mounted with gum.