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Immunohistochemical staining

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Protocol status: Working
We use this protocol and it's working

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Troubleshooting

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

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).

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).

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

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

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

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

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

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

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.

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

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

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

14 Slides are washed with running water for 6 min.

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).

16 Cover slides are mounted with gum.