

immunohistochemistry protocol

Let slides dry at room temperature for 30min

Use this time to label slides and create ridges around the tissue sections using a DAKO pen or nail polish (to prevent incubation fluids from escaping).

Deparaffinization

- a. Xylene: 5 x 5 minutes
- b. 100% ethanol: 2 x 5 minutes
- c. 90% ethanol: 5 minutes
- d. 80 % ethanol: 5 minutes
- e. 70 % ethanol: 5 minutes
- f. Washing TBS/PBS buffer 3×5min
- g. Sections were immersed in normal goat serum or 2% BSA for 60 min to block

Antigen retrieval

- a. Sections were pretreated for antigen retrieval by autoclave at 121 °C for 15 min
Heat-induced: Sodium citrate 10 mM, pH 6.
- b. Cool section on bench top for 60 minutes.
- c. Wash sections with ddH₂O twice for 5 minutes each time

Normal blocking

- a. Immerse each section with 100-400 µl of Normal Blocking Solution(normal goat serum or 2% BSA) for 1 hour at room temperature.
- b. Rinse the excess Normal Blocking Solution with a gentle stream of PBS Buffer from a wash buffer bottle and wash section with Washing Buffer for 10 minutes.

Primary incubation

- a. Dilute primary antibody and negative control antibody with Antibody Dilution Buffer at proper working concentration according to manufacturer's guidance.
- b. Add 100-400 µl of diluted primary antibody and negative control antibody to each section and incubate at 37 °C for 1 hour or 4 °C for 12 to 24 hours in a closed incubation chamber.
- c. Rinse the excess antibody from the section with a gentle stream of PBS Buffer from a wash bottle and immerse sections in Washing Buffer twice for 10 minutes at room temperature.

secondary antibody incubation

- a. Dilute HRP antibody with Antibody Dilution Buffer at proper working concentration according to manufacturer's guidance.
- b. Add 100-400 µl of diluted HRP to each section and incubate at room temperature °C for 30 minutes in an incubation chamber.
- c. Rinse the excess antibody from the section with a gentle stream of PBS Buffer from a wash bottle and immerse sections in Washing Buffer twice for 10 minutes at room temperature.

DAB stain

- a. Add 100-400 μ l of DAB Reagent or other substrate to each section and react for 2 to 7 minutes to stain.
- b. Rinse sections with PBS buffer to stop staining.

Note: The following step is additional but not necessary.

Hematoxylin stain

Counterstain sections in Hematoxylin Reagent according to manufacturer's instructions.

Dehydrate sections

- a. Immerse sections with 95% Ethanol twice for 10 seconds each time.
- b. Immerse sections with 100% Ethanol twice for 10 seconds each time.
- c. Immerse sections with Xylene, incubating sections for 10 seconds each time.

Mount coverslips