Immunohistochemistry (IHC)

I. Preparation of Solutions and Reagents

1. PBS Buffer:Dissolve PBS powder in ddH_2O. Store at room temperature.
2. PBST Buffer:Add Tween-20 to PBS buffer at a ratio of 1 mL Tween-20 per 1 L PBS buffer. Mix thoroughly. Store at room temperature.
3. Citrate Buffer: Mix 54 mL of 0.1 M citric acid solution with 46 mL of 0.1 M sodium citrate solution, then add ddH_2O to a final volume of 1 L. Adjust the pH to 6.0. Store at room temperature.
4. Blocking Solution: This is a 10% goat serum solution. Prepare by mixing 1 mL of goat serum with 9 mL of PBS buffer. Store at –20 °C.

II. Experimental Procedure

A. Slide Baking

Place paraffin tissue sections in a 65°C oven and bake for 3 hours. This removes moisture, enhances tissue adhesion, and prevents detachment of the sections.

B. Deparaffinization and Rehydration

1. Quickly immerse the baked slides in the first container of xylene for 10 minutes.
2. Transfer the slides to a second container of xylene and immerse for another 10 minutes.
3. Immerse the slides in absolute ethanol for 5 minutes.
4. Immerse the slides in 85% ethanol for 5 minutes.
5. Immerse the slides in 70% ethanol for 5 minutes.
6. Rinse the slides under running water until the surface changes from cloudy to clear. During this step, avoid letting the water stream hit the tissue directly; allow the water to flow over the side of the slide to prevent tissue damage.
7. Wash the slides in PBS 3 times, 5 minutes each time.

C. Antigen Retrieval

Heat the citrate buffer solution in a pressure cooker until steam is released evenly from the cooker, indicating that the solution is sufficiently heated.

Place the tissue sections into the boiling citrate buffer. Once the pressure cooker releases steam evenly, continue heating for an additional 2 minutes to retrieve antigens in the tissue.

Allow the pressure cooker to cool naturally. Remove the container holding the sections and gently cool the container with cold water. Avoid rapid cooling to prevent tissue detachment from the slides.

D. Quenching Endogenous Peroxidase Activity

Incubate the tissue sections in 3% hydrogen peroxide solution for 10 minutes to eliminate endogenous peroxidase activity.

Rinse the sections with PBS three times, 5 minutes each time.

E. Blocking

1. Use a hydrophobic barrier pen to circle the tissue. Immediately add PBST solution after drawing the circle to prevent the tissue from drying out.
2. Remove the PBST solution, then add blocking buffer to the tissue section (100–300 µL per slide). Incubate at room temperature for 1 hour.

F. Primary Antibody Incubation

1. Prepare the primary antibody dilution using the blocking buffer (refer to the product website for recommended dilution ratios).
2. Remove the blocking buffer, then add the primary antibody dilution to the tissue. Incubate overnight at 4°C, or for 2–3 hours at room temperature.
3. Wash with PBS three times, 5 minutes each. (If incubated at 4°C, allow the slides to warm to room temperature for 40 minutes before washing to prevent tissue detachment.)

G. Secondary Antibody Incubation

1. Remove the HRP-conjugated secondary antibody working solution (specific to the host species of the primary antibody) from 4°C storage and allow it to return to room temperature.
2. Apply the secondary antibody to the tissue section and incubate at room temperature for 30 minutes.
3. Wash with PBS three times, 5 minutes each.

H. Color Development

1. Prepare the DAB working solution and apply 100–300 µL to the tissue section. Incubate for 1–3 minutes.
2. During DAB development, continuously monitor the color change. Once the tissue appears brownish-yellow with appropriate intensity, immediately immerse the section in distilled water to stop the reaction.

I. Hematoxylin Staining

1. Immerse the slide in hematoxylin staining solution. The required staining time varies depending on the type of hematoxylin used, ranging from a few seconds to several minutes. It is recommended to start with a few seconds and adjust the time based on the staining outcome.
2. After staining, immediately rinse the slide under running water to remove excess dye (avoid directing the water flow directly onto the tissue).

J. Dehydration and Clearing

1. Immerse the slide in 70% ethanol for 3 minutes.
2. Immerse the slide in 85% ethanol for 3 minutes.
3. Immerse the slide in absolute ethanol for 3 minutes.
4. Immerse the slide in the first xylene solution for 2 minutes.
5. Immerse the slide in the second xylene solution for 2 minutes.

K. Mounting

1. After the surface of the slide has air-dried, apply a drop of neutral balsam mounting medium onto the tissue to mount the cover slip (take care to avoid creating air bubbles during the process).
2. Observe the stained tissue under a microscope and photograph to document the experimental results.

III. Experimental Precautions

1. Deparaffinization with xylene:The immersion time should be adjusted according to room temperature and the freshness of the xylene. Higher room temperatures and fresher xylene require shorter immersion times. The operation should ensure that no paraffin residues remain on the tissue.
2. Common antigen retrieval methods: These include citrate heat-induced epitope retrieval (HIER), EDTA HIER, and enzyme digestion.

-Citrate-based retrieval generally results in a cleaner background but may lead to incomplete retrieval for certain nuclear antigens, causing weak or absent immunostaining signals. In such cases, EDTA retrieval can be used as an alternative.

-However, strong EDTA retrieval may sometimes increase background staining.

-For tissues with abundant extracellular matrix, long fixation times, high cross-linking levels, or poor responsiveness to heat-induced retrieval, enzyme digestion may be more suitable.

-The choice of antigen retrieval method should be based on the nature of the antigen, fixation condition of the tissue, and the characteristics of the antibody.

3. Endogenous peroxidase blocking

For tissues with high endogenous peroxidase activity, consider prolonging the incubation time with 3% hydrogen peroxide or increasing the concentration of the hydrogen peroxide solution.

IV. Recommended Experimental Products

1. Antibody: https://www.targetmol.com/all-antibodies
2. Tween 20, T9526

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