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Immunohistochemistry Guide for Slide-mounted Paraffin Sections

Deparaffinizing and rehydration

1. Immerse the slides in Xylene Ⅰ and Xylene Ⅱ successively for 10 minutes respectively.
2. Immerse the slides in anhydrous ethanol Ⅰ , anhydrous ethanol Ⅱ , 95% ethanol, 80% ethanol, and 70% ethanol successively for 5 minutes respectively, and then use deionized water to wash the slides for 2 minutes and repeat 2 times.

Antigen retrieval (optional)

3. Put the slides into the repair box, and then add 0.01M citric acid buffer (PH6.0) to make the tissue immersed.
4. Repair the antigen with medium power microwave for 10 minutes (start timing when the liquid boils), and avoid the tissue from drying during the process.
5. Take out the repair box from microwave oven, and it cool down naturally. Take out the slides when the repair solution cooled down to room temperature, and wash the slides with PBS (PH7.4) for 3 minutes and repeat 3 times. (Don't flush the tissue directly during the washing process in order to avoid breaking up the tissue).

Inactivation

6. Add the prepared 3% H_2O_2 to the slides drop by drop to block endogenous peroxidase. Then incubate them at room temperature for 15 minutes (dilute 30% H_2O_2 with methanol or distilled water), finally use PBS to wash the slides with PBS for 3 minutes and repeat 3 times.

Antibody incubation

7. Blot up absorbent paper with PBS, and add 5% normal serum (Sharing the same or similar species with secondary antibodies) drop by drop on the sections, then block it at 37°C for 30 minutes.
8. Wipe dry the liquid around the tissue on the slides with absorbent paper, and use an oil pen to draw a circle around the tissue, and then add the diluted primary antibodies drop by drop. Add PBS to the section of controls if the negative controls are required. After adding primary antibodies, put the slides into wet box to be incubated at 4°C overnight. (The optimum dilution ratio of the antibodies should be pre-determined through experiments in advance).
9. Wash the slides with PBS for 3 times, each time for 2 minutes, and then add HRP-conjugated secondary antibodies after wiping dry the slides with absorbent paper, finally incubate the slides at 37°C for 30 minutes.

Signal detection

10. Wash the sections with PBS for 3 minutes and repeat 4 times, and wipe dry the sections with absorbent paper, then add DAB substrate reagent that is prepared freshly drop by drop to each section, and observe them under a microscope. The positive signal appears brown-yellow or brown in color. The time should be well controlled to avoid the color appears too deep. Wash the section with tap water to terminate the reaction.
11. Hematoxylin counterstaining: Immerse slides in Harris hematoxylin solution for about 30 seconds to 1 minute, and then transfer slides into ethanol solution with 1% HCl after washing with water, finally wash them with water. (Optional)

Dehydration and mounting

12. Firstly immerse the slides in water and wash them, and then put the slides into the following reagents in order to dehydrate and permeate: 70% ethanol, 80% ethanol, 90% ethanol, 95% ethanol, anhydrous ethanol Ⅰ , anhydrous ethanol, Xylene Ⅰ and Xylene Ⅱ . Put the slides in each reagent for 2 minutes, and finally air dry the sections in the fume cupboard.
13. Drop resinene beside the section, and then cover them with the cover glass. In order to avoid air bubble, firstly lay one side of the cover glass flat and then gently lay another side flat. Finally dry the sealed sections by laying them in the fume cupboard
14. Observe the dried sections and collect images with a microscope.