Immunohistochemistry (IHC) Protocol

Paraffin section immunohistochemical experiment

(For scientific research purposes only, not for clinical diagnosis!)

Experimental Procedure

1. Paraffin sections dewaxing to water: Put the sections into environmentally friendly dewaxing solution Ⅰ 10min- Environmentally friendly dewaxing solution Ⅱ 10min- Environmentally friendly dewaxing solution Ⅲ10min- anhydrous ethanol Ⅰ 5min- anhydrous ethanol Ⅱ 5min- anhydrous ethanol Ⅲ5min- distilled water in turn.
2. Antigen repair: The repair is shown in the table above. During this process, the buffer should be prevented from excessive evaporation and should not be dried. After natural cooling, the slide was placed in PBS (PH7.4) and washed by shaking on the decolorizing shaker for 3 times, 5min each time. (Repair fluid and repair conditions are determined according to the tissue)
3. Blocking endogenous peroxidase: The slices were placed in 3% hydrogen peroxide solution, incubated at room temperature away from light for 25 min, and the slides were placed in PBS (PH7.4) and washed three times on a decolorizing shaking table for 5min each time.
4. Serum closure: The tissue was uniformly covered with 3%BSA in the tissue chemical circle and closed at room temperature for 30min. (Primary antibody of goat origin is blocked with rabbit serum, other sources are blocked with BSA)
5. Add primary antibody: Gently shake off the sealing solution, add PBS to the section in a certain proportion of primary antibody, and the section is placed flat in a wet box at 4°C for overnight incubation.
6. Adding secondary antibody: The slide was placed in PBS (PH7.4) and washed by shaking on the decolorizing shaker for 3 times, 5min each time. After the slices were slightly dried, the tissue was covered with the secondary antibody (HRP label) of the corresponding species of the primary antibody, and incubated at room temperature for 50min.
7. DAB color development: The slide was placed in PBS (PH7.4) and washed by shaking on the decolorizing table for 3 times, 5min each time. After the sections were slightly dried, the freshly prepared DAB color developing solution was added into the circle. The color developing time was controlled under the microscope. The positive color was brown and yellow, and the sections were rinsed with tap water to terminate the color development.
8. Restaining nuclei: hematoxylin restaining for about 3min, washing with tap water, hematoxylin differentiation solution for a few seconds, rinse with tap water, hematoxylin return to blue solution, and rinse with running water.
9. Dewatering and sealing: Put the slices into 75% alcohol for 5min--85% alcohol for 5min--anhydrous ethanol for 5min-- anhydrous ethanol for 5min-- n-butanol for 5min-- xylene for 5min to dehydrate and transparent, take the slices out of xylene to dry slightly, and seal the slices with glue.
10. Microscopy: The results are interpreted under a white light microscope.