Protocol for Immunohistochemistry Kit

Instrument &Equipment

Pipette, Immunohistochemical pen, Microwave oven or Pressure cooker, Timer, Incubating wet boxes, Slicing frame, Cover glass, Microscope, Bottle, etc.

Solution preparation

The preparation of PBS buffer.

EDTA antigen repair solution and DAB coloring solution preparations are described in this manual.

Experimental temperature: 15-28˚C

Experimental steps

1. Deparaffinization and rehydration

1) Immerse the slices in Xylene for 10 min × 2 times.
2) Removing excess liquid, immerse the slices in anhydrous ethanol for 3 min × 2 times.
3) Removing excess liquid, immerse the slices in 95% ethanol for 3 min.
4) Removing excess liquid, immerse the slices in 85% ethanol for 3 min.
5) The deionized water rinses the slides for 1 min.
6) PBS buffer rinses the slices for 3 min ×3 times.

2. Antigen retrieval (reagent 1, microwave for antigen retrieval)

1) After deparaffinization and rehydration, put the slice on a high temperature resistant plastic slicing frame and then put the slicing frame in the beaker (or repair box).
2) Add the proper amount of 1 × EDTA antigen repair solution (Reagent 1, diluted 20 times with purified water) to the beaker. Make sure that the antigen repair solution immersed the slice.
3) Microwave oven heat with a high temperature to boil the liquid, then adjust to the middle to repair the antigen for 20 min, in this process we must make sure that the slice is immersed in the antigen repair solution.
4) Take the beaker out of the microwave oven and put it in cold water to cool down.
5) When the repair solution is reduced to room temperature, remove the slices and rinse with PBS (pH7.4) for 3 min × 3 times.

Notices:

1) During the antigen repairation, make sure that the slice is immersed in the antigen repair solution.
2) The amount of the repair fluid is 800 mL/1 frame -1500 mL/3 frames.
3) When removing the slices and rinse with PBS, do not rinse the tissue directly to avoid breaking the tissue.

3. Inactivation of Endogenous peroxidase

1) Dry the slice with absorbent paper, and paint around the tissue with an immunohistochemical pen.
2) Add 100 μL 3% H_2O_2 (Reagent 2) to slice to inactivate endogenous peroxidase, incubate for 15 min at room temperature.
3) PBS buffer rinses the slices for 3 min × 3 times.

4. Blocking

Dry the slice with absorbent paper. Add Normal goat serum (Reagent 3), incubate for 15 min at room temperature to reduce non-specific staining.

5. Incubation of primary antibody

1) Dry the slice with absorbent paper.
2) Add 100 μL Reagent 4 to the slice, for the negative control experiment, PBS replace the primary antibody should be added to the slice. Incubate in a wet box at room temperature for 1 h or 4℃ overnight.

6. Rewarming

1) If the slice was incubated with primary antibody at 4℃ overnight, take out of the slice from the refrigerator next morning and incubate at room temperature for 15 min. If the slice was incubated with primary antibody at room temperature for 1 h, rinse the slice according to the next step.
2) PBS buffer rinses the slice for 3 min ×3 times.

7. Add Polymer Helper (Reagent 5)

1) Dry the slice with absorbent paper, add Reagent 5 (Polymer Helper), incubate at room temperature or 37 ℃ for 20 min.
2) PBS buffer rinses the slice for 3 min × 3 times.

8. Incubation of secondary antibody

1) Dry the slice with absorbent paper, add Reagent 6 (Polyperoxidase-anti-mouse/rabbit IgG), incubate at room temperature or 37℃ for 20 min.
2) PBS buffer rinses the slice for 3 min × 4 times.

9. Signal detection

1) Get rid of PBS buffer, dry the slice with absorbent paper.
2) Add 100 μL freshly prepared DAB solution (Reagent 7: Reagent8=1:49) to each slice, incubate for 3-5 min, observe the staining results under the microscope, don't make the color too dark.
3) Flushing the slice with water to terminates the coloration.

10. Counterstaining

Add 100 μL Hematoxylin solution (Reagent 9) to the slice, incubate for 30 s-1 min. Flush the slice with water for 5 min to terminate the Counterstaining.

Notices:

Since hematoxylin counterstaining and the length of the incubation time are different, the staining reaction of the nucleus from light blue to the dark blue, over or weak staining will influence the judgment of the correct result.

11. Dehydration and mounting

1) Immerse the slices in 70% ethanol for 2 min.
2) Immerse the slices in 80% ethanol for 2 min.
3) Immerse the slices in 90% ethanol for 2 min.
4) Immerse the slices in anhydrous ethanol for 2 min × 2 times.
5) Immerse the slices in Xylene for 2 min × 2 times.
6) Drop resinene beside the tissue, and then cover it with the cover glass.

Result judgment

1. The results of immunohistochemical examination need to be observed and judged under the microscope.
   The results of immunohistochemical staining must be established on the basis that the positive control gets a positive result and the negative control gets a negative result. The result of the experiment slice should be positive (+) or negative (-).
   Negative staining results showed no brown staining in the tissues.
   Notice: In each immunohistochemical experiment process, positive control and negative control must be applied, otherwise the results are not credible.

2. If the positive control gets a positive result and the negative control gets a negative result, the test slice gets a positive staining showed that there was target antigen in the tissue.

3. If the positive control gets a positive result and the negative control gets a negative result, the test slice gets no positive staining showed that the possibility of expression target antigen in the tissue was low.

4. If both of the positive control and the negative control get negative results, it should be the reagent fails or the test operation is wrong and we have to redo the experiment.