Manual IHC Staining Procedure

1.Baking slice

The tissue sections were placed in a drying oven at 65℃ for 1 hour.

2.Deparaffinization and hydration

Place tissue sections in xylene and soaked twice, 10min/ time. Immerse in 100%, 95% ethanol ,85% ethanol ,75% ethanol for 3min, respectively. Rinse with distilled water for 3min.

3.Antigen retrieval: Use induction cooker to heat antigen retrieval buffer II in the pressure cooker to boiling. The deparaffined and hydrated slices were placed on a heat-resisting staining holder and put into the pressure cooker. Cover the pot and continue heating until spray steam. After 2.5min, remove the pressure cooker from heat source and cools down naturally for 5min. Continually cool down with tap water, remove the valve and open the cover. Remove the slice out from liquid in the pot after it cools down to room temperature naturally. Soak in distilled water was twice, 5min/ time.

Note: During antigen retrieval, the amount of retrieval solution must ensure that the slices are always immersed in the liquid.

4.Add endogenous peroxidase blocking reagent

Rinse the sections with TBS solution for 2 times. The test tissue area on the slide was defined with an oil pen. Remove the solution, added 100μL of endogenous peroxidase blocking reagent to the defined area and incubate at room temperature for 5min. Rinse and soak with TBS solution for 3 times, 3min/ time.

5.Add primary antibody

Remove TBS solution, add 100μL primary antibody, and incubate at room temperature for 60min. Rinse and soak with TBS solution for 3 times, 3min/ time.

6.Add secondary antibody

Remove TBS solution, add 100μL enzyme-labeled polymer, and incubate at room temperature for 30min. Rinse and soak with TBS solution for 3 times, 3min/ time.

7.Chromogen

Remove TBS solution, and add 100μL fresh DAB solution, incubate for 5min. Rinse with purified water for 3 times, 3min/ time.

Note: Fresh DAB staining solution: prepared by 1:20 ratio of DAB substrate and DAB buffer solution.

8.Counterstain

Remove purified water and incubate with hematoxylin for 2min. The rinse with purified water for 2 times, 5min/ time.

9.Dehydration and transparency

Routine dehydration of gradient ethanol, transparentize with xylene.

10.Sealed with neutral balata and coverslip.

Note: The amount of DAB staining solution reagent needs to be added according to the size of the experimental tissue section. The general principle is to cover the tissue with reagents, but not too much.

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