Immunohistochemistry（IHC）Detection Kit

Cat.No.: RK05872

Size: 50 T

Product introduction

IHC, also known as immunohistochemistry, is the application of the immunology principle of specific binding of antigens and antibodies. It determines the intracellular antigens (polypeptides and proteins) by chemical reaction to develop the color of the labeled antibodies (fluorescein, enzyme, metal ion, isotope), and carried out localization, qualitative and relative quantitative information. This IHC kit is suitable for paraffin-embedded tissue section, frozen section, cultured cell smear, and blood samples.

Packing list of the Kit

Cat.No.	Description	Size
RK05872-1	1×PBS Instant Particles	1L/Bag (2)
RK05872-2	Blocking Solutions	5mL
RK05872-3	Antibody Diluents	5mL
RK05872-4	Secondary Antibodies Fluids	5mL
RK05872-5	DAB Reaction Buffer B	5mL
RK05872-6	DAB Reaction Buffer C	50pL
RK05872-7	Hematoxylin	5mL

RK05872-8	$C_6H_8O_7 \cdot H_2O$	1g
RK05872-9	$NaC_6H_5O_7 \cdot 2H_2O$	6g
	Product instruction	1 copy

Storage condition:

Can be stored at 2-8℃ within 10 months.

Instruction

Experiment instruments & reagents

1. Experiment instruments

Microwave, 4℃ fridge, constant temperature and humidity chamber, oven, optical microscope, pipette, antibody incubation box, antigen retrieval box.

2. Experimental reagents

(1) PBS Buffer ：0.01M PBS pH7.2±0.2

Pour 1xPBS buffer powder (1 L) into a clean beaker, then add distilled water (about 700mL) to dissolve.

Adjust pH to 7.4 with HCl, then fill up to 1 L with deionized water in a graduated cylinder.

Mix well and ready to use.

The prepared PBS buffer can be stored at 4C.

(2) PBST Buffer ：0.01M pH7.2 PBST

Reagent	1*PBST/L

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1*PBS buffer	1L
Tween 20 solution	0.001L
Adjust pH with dilute HCl and 2M NaOH solution	

(3) Antigen Retrieval Solution

pH6.0 0.01M Citric Acid Retrieval Solution (1L) :

$C_6H_8O_7.H_2O$ 0.4g ; $NaC_6H_5O_7.2H_2O$ 3g ; pH6.0

(4) 3% Hydrogen peroxide (fresh preparation, 30% H_2O_2 and dH_2O volume ratio 1:9)

(5) Deparaffinization solution, absolute ethanol (analytical grade), deionized water (dH_2O), Mounting Medium.

Experimental procedures

1. Rehydration/Deparaffinization:

(1) Heat slides:

Place paraffin sections on the slice rack in the same direction.

Put these sections in a 55。C oven for 30 min. Meanwhile, place a cylinder of deparaffinization solution 1 in a 55。C oven as well.

(2) Deparaffinization and rehydration:

Place above mentioned paraffin sections in deparaffinization solution 1 with the rack.Remove paraffin sections from the incubator and place at room temperature for 5 minutes.Take the sections out and immerse in deparaffinization solution 2 at room temperature for 5 min, then immerse in deparaffinization solution 3, anhydrous ethanol 1, anhydrous ethanol 2, anhydrous ethanol 3 in order for 5 min each.Rinse the slices with running water for 5 min.

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The water flow should not face the slides during the rinsing process.

Keep the slices moist during operation.

2. Inactivation of Endogenous Peroxidase

Immerse the slices completely in a 3% H_2O_2 solution.

Incubate the slices for 10 min at room temperature.

Rinse the slices with running water for 5 min.

Note: For tissues with high endogenous enzyme content, the inactivation time can be extended to 15-20 minutes; H_2O_2 needs to be freshly prepared, 3% H_2O_2 solution can be prepared 5-10 minutes in advance.

3. Antigen Retrieval (Optional)

Method 1: Microwave antigen retrieval:

Immerse the sections in an antigen retrieval box filled with repair solution, and place its box cover obliquely on the retrieval box。

Put the whole box into the microwave oven, heat it on high heat for 3 minutes, then stop heating and let stand for 5min in the microwave.

Reheat for 3 min with high heat, then stop heating and let stand for 5 min.

Reheat on medium-low heat for 1 min, then stop heating and let stand for 5 min.

Take out the sections along with whole antigen retrieval box and slowly cool down to room temperature.

Once the repair fluid cools to room temperature, rinse slices with PBS buffer for 3 times, 1 min each time.

Method 2: High-pressure antigen retrieval:

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Preheat antigen retrieval solution on high heat in a pressure cooker.

Once the repair solution is boiling, immerse the slices and soak the tissue completely; Cover the pot, close the pressure valve, and continue to heat on high heat.

Switch to medium heat when the pressure limiting valve starts to rotate.

Leave the heat source for 2 min, and move the pressure cooker into cold water to cool slowly;

After the temperature of the retrieval solution is down to room temperature, rinse with PBS buffer for 3 times, 1 min each time.

Note:

The tissue on the section should be immersed in the retrieval solution completely;

During the retrieval process, it is strictly forbidden to open or interrupt the instrument;

Avoid rapid cooling after the retrieval process is completed;

The repair solution can be selected according to the experimental needs.

4. Staining:

(1) Blocking:

After the repair solution has cooled to room temperature, remove the slices from the retrieval box.

Rinse the slices with PBS buffer twice, 3min each time.

Remove the buffer; Drop blocking solution on the tissue sections.

Place the slices horizontally in an antibody incubation box with water at the bottom, and incubate at 37°C for 30 min.

(2) Primary antibody incubation:

Remove the blocking solution, drop the primary antibody diluted with antibody dilution

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buffer on the tissue section, place in an antibody incubation box horizontally and incubate overnight at 4°C.

(3) Rewarming: Take out the antibody incubation box and rewarm for 15-30min at room temperature; Remove the antibody solution, and rinse with PBST buffer once for 5min; Rinse with PBS buffer for 3 times, 5min each time.

(4) Secondary antibody incubation: Drop the secondary antibody solution on the tissue section, place in an antibody incubation box horizontally and incubate at 37°C for 1 h.

(5) Remove the solution on the slices, rins with PBST buffer once for 5 min; Rinse with PBS for 3 times, 5 min each time.

(6) Staining: 5min before staining , prepare DAB chromogen solution by mixing solution C and solution B well at a volume ratio of 1:100 ; Drop the chromogen solution on the tissue section, observe the color change closely under the microscope, usually get the appropriate staining intensity in 10-60 seconds; Stop staining by immersing slices in large amount of dH_2O.

(7) Counterstaining and bluing: Immerse the slightly drained sections in counterstaining reagent (hematoxylin) to counterstain the sections, and rinse with running water for 10 minutes.

Note:

During staining process, keep the sections moist all the time, and ensure that the tissue on the sections is completely covered with the reagents used in the process;

The water flow should not be directed to face the slides during the rinsing process, otherwise it may cause the slices to fall off.

5. Dehydration and mounting:

(1) Dehydration: Immerse the cleaned slices in absolute ethanol once for 10 sec;

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Completely dry at high temperature (55℃-60℃).

(2) Mounting: Drop an appropriate amount of Neutral Balsam in the center of the slice and cover with a coverslip.

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