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S-vision immunohistochemistry kit (anti-mouse secondary antibody)

Product Number: S110053

Shipping and Storage

Wet ice for transport; store at 2-8°C with a 12 month shelf life.

Component

Component	S110053-100T
S-vision immunohistochemical polymeric secondary antibody (goat anti mouse)	10mL
50×DAB original solution	250μL
DAB diluent	12.5mL

Description

This kit is a highly sensitive two-step immunohistochemistry reagent kit, suitable for subsequent color development with primary antibodies derived from mice. The enzyme-labeled secondary antibody in the kit consists of a polymer formed by conjugating multiple HRP enzyme molecules and antibody molecules to a dextran backbone. This polymer enhances the enzyme labeling density of the secondary antibody, creating a highly sensitive, ready-to-use, one-step non-biotin detection system for immunohistochemistry and immunocytochemistry staining. It exhibits broader adaptability to tissue fixation methods and fixation times as well as tissue section storage durations, reducing variability caused by differences in tissue collection and handling methods. The kit features simplicity, speed, high sensitivity, excellent reproducibility, and biotin-free interference. The secondary antibody reagent is ready-to-use and does not require dilution to achieve optimal staining ratios, minimizing experimental operational errors. With 100μL of secondary antibody and chromogen solution per sample, this kit can test up to 100 samples.

Protocol

1. Usage - Hand Dyeing

The recommended routine immunohistochemical staining process is as follows:

1.1. Place the slices in environmentally friendly dewaxing solution I for 10 minutes, environmentally friendly dewaxing solution II for 10 minutes, environmentally friendly dewaxing solution III for 10 minutes, anhydrous ethanol I for 5 minutes, anhydrous ethanol II for 5 minutes, anhydrous ethanol III for 5 minutes, and then rinse with distilled water;

1.2. Microwave repair: Heat to boiling and maintain a high temperature of 98°C or above for 15-20 minutes, then cool naturally to room temperature; High pressure repair: Heat the high-pressure cooker until evenly sprayed, and time for 2 minutes.

Note: The repair time is related to the sample fixation time and antigen epitope, and conditions can be explored.

1.3. Soak the slices or drip until completely covering the tissue, and incubate at room temperature for 10 minutes. Place the glass slide in PBS (pH 7.4) and wash it three times on a decolorization shaker, each time for 5 minutes;

1.4. Prepare a 3% BSA working solution and add it dropwise until it completely covers the tissue. Incubate at room temperature for 30 minutes;

1.5. Drop the primary antibody working solution until it completely covers the tissue, usually 100μL, and incubate overnight at 4°C or for 2 hours at 37°C;

1.6. Add immunohistochemical polymeric secondary antibody dropwise until completely covering the tissue, usually 100μL, and incubate at room temperature for 20 minutes;

1.7. Prepare the DAB color reagents (reagent 2 and reagent 3) in a 1:50 volume ratio to form the working solution. Drop the prepared DAB working solution until it completely covers the tissue, and develop the color for 5-10 minutes;

1.8. Stain with hematoxylin solution for 1-3 minutes, then wash with water; Differentiate with hematoxylin differentiation

solution for several seconds and wash with water; Return the hematoxylin blue solution to blue for 1 minute and wash with water;

1.9. 75% alcohol for 5 minutes -85% alcohol for 5 minutes - anhydrous ethanol I for 5 minutes - anhydrous ethanol II for 5 minutes - n-butanol for 5 minutes - xylene I for 5 minutes, dehydrate and make transparent. Remove the slices from xylene and air dry them slightly. Seal the slices with adhesive.

2. Automation in IHC

2.1. Dewaxing solution at 62℃ for 3 minutes, repeated 3 times, washed 3 times with anhydrous ethanol, and washed 2 times with pure water;

2.2. Special repair solution for chemical analyzer, 25 minutes at 100℃, cooled for 20 minutes;

2.3. 3% hydrogen peroxide, 100-150μL, room temperature for 10 minutes;

2.4. 3% BSA working solution, 100-150μL, room temperature for 10 minutes;

2.5. Primary antibody working solution, 100-150μL, incubated at 37℃ for 15-30 minutes, washed 3 times with PBS;

2.6. 100-150μL of secondary antibody working solution, incubated at room temperature for 20 minutes, washed 3 times with PBS;

2.7. Prepare the working solution for reagent 2 and reagent 3 in a volume ratio of 1:50. Use 100-150μL of DAB color developing working solution for 5-10 minutes, and wash with pure water three times;

2.8. Mayer hematoxylin staining solution for 10 minutes, washed three times with pure water;

2.9. 75% alcohol for 5 minutes -85% alcohol for 5 minutes - anhydrous ethanol I for 5 minutes - anhydrous ethanol II for 5 minutes - n-butanol for 5 minutes - xylene I for 5 minutes, dehydrate and make transparent. Remove the slices from xylene and air dry them slightly. Seal the slices with adhesive.

Note

1. Reagent 1 is a ready-to-use without dilution.Reagents 2 and 3 are diluted in a 1:50 volume ratio.
2. If the background is deep, the primary antibody can be diluted by gradient to obtain better results.
3. The quantity of this kit is calculated according to the manual staining method. If use in the histochemical instrument, you need to calculate the dosage ratio of each component according to the steps of the instrument or directly purchase the special reagent set for the histochemical instrument.
4. Wear protective equipment. for your safety.