DAB Horseradish Peroxidase Color Development Kit

Product description

Diaminobenzidine (3,3'-diaminobenzidine, DAB) is a common chromogenic substrate of horseradish peroxidase (HRP), as an HRP electron donor, loses electrons in the presence of hydrogen peroxide, rapidly changes color, and forms insoluble brown or amber particles through polymerization and other reactions, and settles in situ. This brown precipitate is insoluble in water and ethanol, so after DAB color development, subsequent staining can also be performed using an ethanol-soluble dye.

This kit is a horseradish peroxidase (HRP) substrate detection kit that can be used for immunohistochemistry, in situ hybridisation, protein blotting, spot blotting and other experiments.

Components and storage conditions

Components	K4152-20 mL	K4152-100 mL
DAB Reagent A	10 mL	50 mL
DAB Reagent B	10 mL	50 mL
Store the components at -20°C away from light for 12 months.		

Experimental manipulation

1. Sample handling - processing of tissue sections/cell samples/membranes

For tissue sections or cell samples or membranes, after incubation with horseradish peroxidase-labeled antibodies or other forms of probes, select appropriate wash solution for 3-5 min each. For tissue or cell samples that detect endogenous horseradish peroxidase, after sample fixation, also wash 3-5 times with appropriate wash solution for 3-5 min each.

2. Configure DAB staining working solution

Components	1 mL	10 mL
DAB Reagent A	0.5 mL	5 mL
DAB Reagent B	0.5 mL	5 mL
**Note: Mix A and B in equal proportions (v/v) and configure according to the required amount.*		

3. Chromogenic reactions

After the last wash in step 1, remove the wash solution and add appropriate amount of DAB staining working solution to ensure that it can fully cover the sample. Incubate at room temperature away from light for 3~30

min until the colour develops to the desired shade.

4. Termination of chromogenic reactions

The chromogenic reaction can be terminated by removing the DAB staining solution followed by 1-2 washes with distilled water.

5. Post-processing

For tissue sections or cell samples, after the chromogenic reaction is terminated, it can be stained with Neutral Red Staining Solution (K 1185 Neutral Red Staining Solution) if necessary for easy observation. For membranes, after the chromogenic reaction is terminated, it can be dried at room temperature and stored in the dark.

FAQs

1. The background color rendering is too dark

a) If the background color is too dark during immunohistochemistry, on the one hand, it is necessary to consider using an appropriate blocking solution, such as purchasing an appropriate blocking solution or using serum from the same source as the primary antibody (10%). On the other hand, please pay attention to the purchase of secondary antibodies that have undergone proper adsorption to reduce the non-specific adsorption of secondary antibodies.

b) If the background color is too dark during immunohistochemistry, care should be taken to inactivate endogenous catalase. 1 volume of 3% hydrogen peroxide can be added to 4 volumes of methanol, mixed well for the inactivation of endogenous catalase.

c) Shortening the chromogenic time or reducing the secondary antibody concentration may be considered. In addition, choosing the appropriate strength of the washing liquid or extending the washing time can also help.

2. No or weak color rendering

a) Appropriate increases in the concentration of primary or secondary antibodies may be considered. To detect the effect of the secondary antibody, drop a drop to dilute the secondary antibody on the membrane to detect whether the secondary antibody can be normally colored.

b) Consider using more sensitive scale-up assays, such as biotin.

c) The chromogenic time can be appropriately extended and additionally determined whether antigen retrieval is necessary for the primary antibody used.

Notes

1. DAB is harmful to the human body, please pay attention to appropriate protection.

2. DAB working fluids are recommended for ready-to-use.

3. This product is for scientific use only.

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