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Instructions for Use of DAB Substrate Kit

Cat No.: C-1010G

Product profile

Diaminobenzidine (DAB) is the most sensitive and commonly used chromogenic substrate for horseradish peroxidase, and the reaction product is a brown precipitate insoluble in water, xy lene and alcohols. This product adopts special formula with high sensitivity, low background, stable storage and easy to use. Suitable for staining and chromogenic reactions in western blotting, immunohistochemistry and immunocytochemistry, spot blots and biochips, etc.

Packing specifications:

Component	200T
DAB concentrate (50X)	250μL
DAB diluent	12.5 mL

Usage method:

1. Prepare 1x DAB color working solution before use: add 20 μL DAB of concentration (50X) to every 1mL DAB of dilution, mix well and reserve. Need to be ready for current use, limited to the same day of use, long-term preservation is not recommended. Note: Do not use a buffer containing sodium azide, an HRP inhibitor.

2. After the last washing of the sample, remove the washing solution and add enough DAB color working solution to ensure adequate coverage of the sample; incubate at room temperature for 3-30 min or longer until the desired depth. Immunoblot experiments should ensure that the sample is in an enough staining solution and can be freely shaken.

3. For immunohistochemistry or in situ hybridization, using 50 μ L of color solution for each sample, this kit can detect a minimum of 200 samples.

4 The DAB staining working solution was removed and the samples were rinsed several times with PBS to abort the chromoreaction.

5. For the cell or tissue samples observed with the microscope, the samples can be counterstained (optional) and mounted in the aqueous sealing media. Alternatively, the sample may be dehydrated and mounted in organic sealants. For immunoblotting experiments, samples can be rinsed with water, air dried and then stored at room temperature.

Storage and transportation:

2-8℃Closed storage away from light, effective for one year. Avoid repeated freezing and thawing.

Matters need attention

1. DAB is harmful to human body. Please be careful during operation and take effective protection to avoid direct contact or inhalation into human body.

2. Ensure that all reagents are completely melted and mixed before use.

3. The color rendering working solution should be used now, and the freshly prepared working liquid should be colorless or light brown, if the color is too dark, do not use.

4. Strictly control the color rendering time, and adjust it according to the situation to avoid excessive color rendering. The solid phase membrane will fade after several hours and cannot remain permanent.

5. Harmless treatment: please soak the container stained with DAB color rendering solution in the solution containing 3% KMnO4 and 2% Na HCO 3 for 3h to reduce contamination.

6. This product is limited to professional scientific research purposes, and shall not be used for clinical diagnosis or treatment, or for food or medicine,

It shall not be stored in an ordinary residence.

7. For your safety and health, please wear laboratory clothes and disposable gloves.

Common questions and analysis

1. The background color is too dark.

A. If the background is too dark, consider using the appropriate blocking solution for sealing, such as choosing the appropriate blocking solution or using the serum of the same source (10%) as the primary antibody. On the other hand, please pay attention to the purchase of properly adsorption secondary antibodies to reduce the non-specific adsorption of secondary antibodies.

B. If the background is too dark, attention should be paid to inactivate endogenous catalase.

C. Reduce the color our time, or reduce the secondary antibody concentration.

D. Select the washing solution of appropriate strength, or extend the washing time.

2. No color development or too weak color development.

A. Appropriate increase in the concentration of primary or secondary antibodies may be considered. To test the effect of the secondary antibody, a drop of the secondary antibody was diluted on the membrane to test whether the secondary antibody can be normally colored.

B. Consider using a more sensitive amplification detection system, such as the biotin detection system.

C. Chromogenic can be appropriately extended to additionally determine whether antigen repair is necessary for the primary antibody used.