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Diaminobenzidine (DAB) Histochemistry Kits

D22185 Diaminobenzidine (DAB) Histochemistry Kit #1 *with goat anti–mouse IgG–HRP*
D22187 Diaminobenzidine (DAB) Histochemistry Kit #3 *with streptavidin–HRP*

Quick Facts

Storage upon receipt:

- ≤ –20°C
- Desiccate
- Protect from light

Introduction

The use of horseradish peroxidase (HRP) for enzyme-mediated immunodetection, commonly referred to as immunoperoxidase labeling, is a well-established histochemical technique.[1-3] The most widely used HRP substrate for these applications is diaminobenzidine (DAB), which generates a brown-colored polymeric oxidation product. The DAB reaction product is discretely localized at HRP-labeled sites, providing high resolution images of subcellular antigen distribution. DAB staining can be visualized directly by bright-field light microscopy or, following osmication, by electron microscopy. Molecular Probes offers DAB Histochemistry Kits for detection of mouse IgG primary antibodies (D22185) and biotinylated antibodies or tracers (D22187).

Materials

Contents

- **3,3′-Diaminobenzidine, tetrahydrochloride, dihydrate** (DAB; Component A), 50 mg (note **A**).
- **HRP–conjugated secondary antibody or streptavidin conjugate** (Component B), 100 µg
- **Blocking Reagent** (Component C), 4 g
- **Staining Buffer** (Component D), 55 mL
- **Hydrogen peroxide** (H_2O_2, Component E), 300 µL of a 30% stabilized solution

Each kit provides sufficient materials to stain 200 slide preparations.

Storage

Upon receipt and prior to use, store the kits at ≤–20ºC, desiccated and protected from light.

Applications Protocols

Preparation

1.1 Prepare phosphate-buffered saline (PBS) (not provided) according to standard laboratory protocols.

1.2 Prepare a 10 mg/mL DAB stock solution by dissolving the 50 mg of solid material provided (Component A) in 5 mL of Staining Buffer (Component D). Mix by swirling or gentle vortexing until the DAB powder has gone into solution. Filter the DAB solution through a 0.2 µm syringe filter. Divide the filtered DAB stock solution into small aliquots and store frozen at ≤–20ºC.

1.3 Prepare a 500 µg/mL stock solution of the HRP conjugate stock solution by reconstituting the material provided (Component B) in 200 µL of PBS. This solution may be stored at 4°C for up to 3 months if required. Optionally, add 0.02% thimerosal as a preservative. Note that sodium azide must NOT be used for this purpose.

1.4 Prepare a 1% (10 mg/mL) solution of Blocking Reagent in PBS. We recommend preparing only as much as is needed for immediate use. However, unused solution can be stored frozen at ≤–20°C for 1 month if necessary.

Peroxidase Labeling

2.1 Fix cell or tissue specimens following customary procedures.

2.2 If necessary, quench endogenous peroxidase activity by incubating the specimen in 1–3% H_2O_2 (diluted into PBS from the 30% solution provided; Component E) for 1 hour.

2.3 Incubate the specimen with 1% Blocking Reagent solution for 60 minutes at room temperature or 37°C.

2.4 Label the specimen with the primary antibody diluted in 1% Blocking Reagent for 60 minutes at room temperature (note **B)**.

2.5 Rinse the specimen three times with PBS.

2.6 Prepare a 1 µg/mL working solution of the HRP conjugate by diluting the stock solution (prepared in step 1.3) 1:500 in 1% Blocking Reagent (note **C**).

2.7 Apply 250 µL of the HRP conjugate working solution to the specimen and incubate for 30–60 minutes at room temperature.

2.8 Rinse the specimen three times with PBS.

Staining Tissue Sections

3.1 Dilute the DAB stock solution (prepared in step 1.2) 1:10 in PBS to a final working concentration of 1 mg/mL. When diluting aliquots of the DAB stock solution that have been stored frozen, it may be necessary to redissolve precipitated DAB by either vortexing or pipeting up and down.

3.2 For slide-mounted sections: Add H_2O_2 to the DAB working solution to a final concentration of 0.03% (1:1000 dilution from the 30% solution provided). Immediately apply about 250 µL of DAB/H_2O_2 working solution to the surface of the slide. Incubate the slide horizontally and visually inspect the section at low magnification under a microscope against a white background to assess the degree of color development. The time required for completion can vary from 10 seconds to 5 minutes.

3.3 For free-floating sections (>20 µm thickness): Add DAB working solution to sections in a staining dish and incubate with gentle agitation for 15 minutes to allow the DAB solution to fully penetrate the tissue section. Then add 0.03% hydrogen peroxide to each well, mix and visually inspect the section at low magnification under a microscope to assess the degree of color development. The time required for completion can vary from 10 seconds to 5 minutes.

3.4 When the DAB staining reaction is complete, as indicated by visual inspection, wash the DAB solution from the slide or staining dish into a hazardous waste disposal container (note **A**).

3.5 Wash the tissue sections extensively with PBS to remove residual DAB. Transfer used wash buffer to a hazardous waste disposal container (note **A**).

Development for Electron Microscopy

After completion of the DAB staining reaction, specimens can be developed for electron microscopy by exposure to osmium tetraoxide (OsO_4). Osmicated DAB reaction products have high electron opacity and are insoluble in plastic embedding media.

4.1 Post-fix the specimen in 1% glutaraldehyde in PBS for 15 minutes. This step eliminates fracturing of the sections when drying on slides after osmication.

4.2 Rinse the specimen five times with PBS.

4.3 Incubate with 1% OsO_4 in PBS for 30–60 seconds.

4.4 Rinse the specimen five times with PBS.

4.5 Mount out of PBS onto gelatinized slides, air dry, dehydrate in an ascending ethanol series, clear in xylene and mount in DPX under a coverslip.

Mounting and Storing Slides

Sections can be mounted in an aqueous mountant, such as Aqua-Poly/Mount (Polysciences Inc., Warrington, PA) or 90% glycerol in PBS, directly after the final buffer wash (step 3.5). Alternatively, sections can be dehydrated in an ascending ethanol series and then cleared in xylene before being mounted under a coverslip in an organic mounting medium such as Permount® (Fisher Scientific, Pittsburgh, PA) or DPX. Allow slides to dry thoroughly before viewing or storing. Slides can be stored horizontally or vertically in a slide box at room temperature.

Visualization by Light Microscopy

Photographic contrast of DAB reaction products can reportedly be increased by inserting a blue-violet bandpass filter (e.g., Schott BG12) in the transmitted light path.[4] Color intensification of DAB staining by addition of nickel or cobalt ions to the staining solution has been widely reported and practiced.[5,6] Nickel enhancement also changes the color of the DAB reaction product from brown to gray–black. Another reported intensification method involves incubating DAB-stained tissue with nitro blue tetrazolium (NBT, N6495), followed by a brief period of illumination under the microscope.[7]

Notes

[**A**] DAB is a hazardous chemical — harmful if swallowed, inhaled or placed in contact with the skin; an irritant to the eyes, skin and respiratory system; and a suspected carcinogen. DAB should be handled with appropriate precautions — if necessary, consult your institution's chemical safety officer or laboratory safety manuals for guidance.[8] Dispose of DAB in accordance with local, state and federal regulations.

[**B**] Optimal dilutions for primary antibodies should be determined empirically or from specifications provided by the supplier.

[**C**] In general, dilutions of the HRP conjugate between 1:50 and 1:200 can be used, depending on the abundance of the target primary antibody.

References

1. Arch Pathol Lab Med 102, 113 (1978); **2.** J Histochem Cytochem 36, 317 (1988); **3.** J Histochem Cytochem 37, 1609 (1989); **4.** J Histochem Cytochem 36, 701 (1988); **5.** J Histochem Cytochem 29, 775 (1981); **6.** J Microscopy 160, 265 (1990); **7.** J Neurosci Meth 44, 217 (1992); **8.** Lunn, G. and E.B. Sansone, *Destruction of Hazardous Chemicals in the Laboratory,* Wiley and Sons (1990).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
D22185	Diaminobenzidine (DAB) Histochemistry Kit #1 *with goat anti-mouse IgG–HRP*	1 kit
D22187	Diaminobenzidine (DAB) Histochemistry Kit #3 *with streptavidin–HRP*	1 kit
N6495	nitro blue tetrazolium chloride (NBT)	1 g

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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