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# Product Information

**3,3′-Diaminobenzidine (DAB) Liquid Substrate System**

Catalog Number **D7304**
Storage Temperature 2–8 °C

## Product Description

The DAB (3,3′-Diaminobenzidine tetrahydrochloride) Liquid Substrate System has been developed for use in immunohistological and immunoblotting procedures as a precipitating substrate for the detection of peroxidase activity. DAB is the immunohistological substrate of choice because it produces an intense brown stain that is easily observed visually. The end product is resistant to alcohol, therefore, a variety of counterstains and mounting media can be used with the DAB Liquid Substrate System. The DAB Liquid Substrate System provides all the chromogen and buffer/peroxide solutions needed to produce a fast and convenient DAB substrate solution. The DAB Liquid Substrate System is not recommended for ELISA (multiwell) procedures.

## Components

The DAB Liquid Substrate System consists of the following reagents:

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| DAB Liquid Buffer (Catalog Number D7429) | 225 mL |
| 10× DAB Liquid Chromagen (Catalog Number D7554) | 25 mL |

The DAB Liquid Substrate System provides reagents sufficient to prepare 250 mL of DAB Substrate Solution.

## Equipment and Reagents Required but Not Provided

- Test Tubes
- Pipette capable of delivering 1 mL
- Graduated cylinder to measure 9 mL
- Whatman® Puradisc 30 syringe filter units, disposable, cellulose acetate, pore size 0.2 μm, (Catalog Number WHA10462701)
- Nickel(II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$, Catalog Number 223387) or Cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$, Catalog Number 202185), 0.3% (w/v) solution for enhancement of tissue stains
- Tris buffered saline (TBS, Catalog Number T5030) for washing

## Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Preparation Instructions

Prepare 10 mL of DAB Substrate Solution by adding 1 mL of 10× DAB Liquid Chromogen (Catalog Number D7554) to 9 mL of DAB Liquid Buffer (Catalog Number D7429). Mix well. For best results, use the solution immediately.

DAB reactions may be enhanced by the addition of $NiCl_2$ or $CoCl_2$ to the DAB Substrate Solution. Add 1 mL of a 0.3% (w/v) metal salt solution to 10 mL of DAB Substrate Solution. The addition of a metal salt to DAB changes the color from brown to black or blue-black.

Occasionally DAB solutions may be hazy. The haziness may be removed by filtering the DAB solution through a 0.2 μm filter.

## Storage/Stability

The product ships on wet ice and storage at 2–8 °C is recommended. The reagents are stable for 12 months after manufacture. See product labels for actual expiration date.

## Procedure

1. Cover tissue sections with 0.2–0.5 mL of the DAB Substrate Solution.
2. DAB is a fast-reacting substrate. Monitor color development carefully during the reaction to prevent overdevelopment and high background. The reaction may be stopped by gently washing the slide in water or TBS.
3. When finished, dispose of any remaining DAB Substrate Solution in a manner consistent with proper hazardous material handling protocols for your institution.

**Troubleshooting**

Background too high

1. Prior to the application of the primary antibody, block the tissue with 10% (v/v) normal serum from the host species of the second antibody.
2. Prior to antibody incubations, block endogenous peroxidase by flooding the slides with a solution of 4 parts methanol to 1 part 3% $H_2O_2$ solution.
3. Decrease the staining time.
4. Titer the conjugate to optimize the working dilution.

No color develops or color is too faint

1. Adjust the concentration of the primary antibody.
2. Adjust the concentration of the secondary antibody.
3. Determine if the enzyme conjugate is active.
4. Consider using an amplifying system such as avidin-biotin.
5. Increase the staining time.
6. Determine if enzymatic treatment (unmasking) of the antigen is required prior to application of the primary antibody.

**References**


1. Nakane, P.K., and Pierce, G.B., Jr., *J. Histochem. Cytochem.*, **14(12)**, 929-931 (1966).
2. Trojanowski, J.Q. *et al*., *J. Histochem. Cytochem.*, **31,** 1217-1223 (1983).
3. DeJong, A.S.H. *et al*., *Histochem. J*., **17(10)**, 1119-1130 (1985).
4. Chu, N.M. *et al*., *J. Histochem. Cytochem*., **37(2)**, 257-263 (1989).
5. Merchenthaler, I. *et al*., "Silver Intensification in Immunocytochemistry", in *Techniques in Immunocytochemistry* (Bullock, G., and Petrusz, P., eds.). Academic Press Ltd. (San Diego, CA: 1989), pp. 217-252.
6. Hsu, S., and Soban, E., *J. Histochem. Cytochem*., **30(10)**, 1079-1082 (1982).




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