

Product Information

3,3'-Diaminobenzidine (DAB) tetrahydrochloride Enhanced Liquid Substrate System

For immunohistology

D3939

Product Description

Diaminobenzidine (DAB) is used in many applications to visualize peroxidase activity.¹⁻⁶ The DAB Enhanced Liquid Substrate System is for use in immunohistology procedures as a precipitating substrate to detect peroxidase activity. DAB is the immunohistology substrate of choice, because it produces an intense brown stain that is easily observed. The end product is resistant to alcohol. Thus, various counterstains and mounting media in alcoholic solutions can be used with the DAB Liquid Substrate System.

The DAB Enhanced Liquid Substrate System is supplied as two reagents, the buffer and the chromogen, which are mixed together immediately prior to use. The DAB Liquid Substrate System provides all the chromogen and buffer/peroxide solutions that are needed to produce a fast and convenient DAB substrate solution.

Several theses⁷⁻⁹ and dissertations¹⁰⁻²⁸ have cited use of product D3939 in their research protocols.

Storage/Stability

The DAB buffer and DAB chromogen solutions are stable for up to 18 months when stored at 2-8 °C in the original containers.

After combining the two components, the resulting reagent is stable for:

- 2 to 4 hours at room temperature
- up to 8 hours at 2-8 °C

During these time periods, the 1× reagent may develop a darker color. However, the reagent performance will not be affected.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

The DAB Liquid Substrate System consists of the following reagents:

- DAB Liquid Buffer Solution A (Component Number D6190): 100 mL
- DAB Liquid Chromogen Solution B (Component Number D6065): 3 mL (packaged in a dropper bottle)

Reagents and Equipment Required but Not Provided

- 0.2 µm filter
- Tris buffered saline (TBS, such as Cat. No. T5030) for washing

Procedure

1. Add 30 µL (one drop) of the DAB Liquid Chromogen Solution to 1 mL of the DAB Liquid Buffer Solution. Mix well. For best results, use the prepared DAB reagent solution immediately.
2. Cover tissue sections with 0.2-0.5 mL of the DAB reagent solution.
3. DAB is a fast-acting substrate. Monitor carefully during the reaction to prevent over-development and high background. The reaction may be stopped by gently washing the slide in water or TBS.

- Occasionally DAB solutions may be hazy. The haziness may be removed by filtering the DAB solution through a 0.2 µm filter.
- When finished, dispose of any remaining substrate solution in a manner consistent with proper hazardous material handling protocols for your institution.

Troubleshooting

Background too high

- Prior to the application of the primary antibody, block the tissue with 10% (v/v) normal serum from the host species of the secondary antibody.
- Prior to antibody incubations, block endogenous peroxidase by flooding the slides with a solution of 4 parts of methanol to 1 part of 3% H₂O₂ solution.
- Decrease the staining time.
- Titer the conjugate to optimize working dilution.

No color develops or color is too faint

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

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D3939dat Rev 03/22 FEB,GCY,MAM

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