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Product Information

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

Catalog Number **D7679**
Storage Temperature 2–8 °C

Product Description

The DAB (3,3′-Diaminobenzidine Tetrahydrochloride) Liquid Substrate Dropper System has been developed for use in immunohistology and immunoblotting procedures as a precipitating substrate for the detection of 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. Therefore, a variety of counterstains and mounting media can be used with the DAB Liquid Substrate Dropper System. The DAB Liquid Substrate Dropper System provides all of the chromogen and buffer/peroxide solutions needed to produce a fast and convenient DAB substrate solution.

Components

The DAB Liquid Substrate Dropper System consists of the following reagents:

DAB Liquid Buffer (Catalog Number D7429)	10 × 9 mL
DAB Liquid Chromagen (10×) (Catalog Number D7554)	10 mL

Equipment Required but Not Provided

Pipette capable of delivering 1 mL

Optional Equipment and Reagents Not Provided

0.2 μm filter

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) for enhancement of tissue stains, in the form of a 0.3% (w/v) stock solution

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.

Procedure

1. Pipette 1 mL of DAB Liquid Chromagen (Catalog Number D7554) into an unused 9 mL dropper bottle of DAB Liquid Buffer (Catalog Number D7429). Mix well. For best results, use the solution immediately.
2. Cover tissue sections with 0.2–0.5 mL of the DAB solution.
3. DAB is a fast-acting substrate. Monitor carefully during the reaction to prevent overdevelopment and high background. The reaction may be stopped by gently washing the slide in water or TBS.
4. DAB reactions may be enhanced by the addition of $NiCl_2$ or $CoCl_2$. Add 1 mL of a 0.3% (w/v) stock solution of either $NiCl_2$ or $CoCl_2$ to 10 mL of DAB working solution. The addition of either $NiCl_2$ or $CoCl_2$ to DAB changes the color from brown to black or blue-black.
5. Occasionally DAB solutions may be hazy. The haziness may be removed by filtering the DAB solution through a 0.2 μm filter.
6. 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

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.
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

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