

Product Information

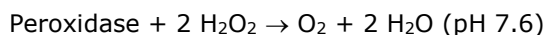
SIGMAFAST™ 3,3'-Diaminobenzidine tablets

Tablet, to prepare 15 mL

D4418**Product Description**

Diaminobenzidine (DAB) is used in many applications for visualization of peroxidase activity.¹⁻⁶ DAB is the immunohistology substrate of choice, as it produces an intense brown-black stain, which is resistant to alcohol. Slides stained with DAB may be cover-slipped in the traditional manner and stored for future reference.

SIGMAFAST™ 3,3'-Diaminobenzidine tablets have been developed for use in immunohistology as a precipitating substrate for the detection of peroxidase activity:



$\text{O}_2 + \text{DAB} \rightarrow$ insoluble, brown-black precipitate

SIGMAFAST™ DAB tablets require no additional ingredients or procedures to prepare an active substrate solution. One SIGMAFAST™ DAB tablet set (one DAB tablet and one Urea Hydrogen Peroxide tablet) dissolved in 15 mL of ultrapure water provides 15 mL of a ready-to-use substrate solution that contains:

- 3,3'-Diaminobenzidine (DAB): 0.7 mg/mL
- Urea Hydrogen Peroxide (H_2O_2 equivalence, 0.7 mg/mL): 2.0 mg/mL
- Trizma® buffer: 60 mM

This product has been used to study such systems as viruses,⁷ honey bee brain,⁸ tissues related to Alzheimer's Disease,^{9,10} sow sera,¹¹ Pertussis toxin samples,¹² cells from cultured mouse embryos,¹³ and rat brain samples.¹⁴ Several theses¹⁵⁻¹⁸ and dissertations¹⁹⁻³² have cited use of product D4418 in their research protocols.

Storage/Stability

Store the tablets at -20 °C.

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

- 3,3'-Diaminobenzidine (DAB) tablets (Component Number D9417): 5SET (5 tablets) or 50SET (50 tablets)
- Urea Hydrogen Peroxide tablets (Component Number U1380): 5SET (5 tablets) or 50SET (50 tablets)

Reagents and Equipment Required but Not Provided

- Ultrapure water (17 MΩ•cm or equivalent)
- Pipette capable of delivering 15 mL
- Test tubes

Additional Optional Materials

- 0.2 µm filter (such as Cat. No. WHA10462701)
- Nickel(II) chloride (NiCl_2) or Cobalt(II) chloride (CoCl_2), to prepare 0.3% (w/v) stock solution to enhance tissue stains
- PBS (such as Cat. No. P4417) for washing

Preparation Instructions

1. Remove the required number of DAB and Urea Hydrogen Peroxide tablets from the freezer.
2. Allow the tablets to reach room temperature.
3. Open DAB tablet package (silver foil) and Urea Hydrogen Peroxide tablet package (gold foil). Drop the tablets into an appropriate container.
Do not touch the tablets with your fingers.

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4. Add 15 mL of ultrapure water.
5. Vortex until dissolved.

The SIGMAFAST™ DAB Substrate Solution is now ready for use. For best results, the solution should be used within one hour.

Procedure

1. Cover the treated tissue section with 0.2-0.5 mL of DAB Substrate Solution.
2. The DAB reaction may occur rapidly. Color development should be carefully monitored during the reaction to prevent overdevelopment and high backgrounds. Reactions may be stopped by gently washing the slide in water or PBS.
3. DAB reactions may be enhanced by the addition of a NiCl₂ or CoCl₂ solution. Add 1.5 mL of 0.3% (w/v) stock solution to 13.5 mL DAB Substrate Solution. The addition of metal salts to DAB changes the color of the precipitate product to black or blue-black.
4. Occasionally the DAB Substrate Solution may be hazy. The haziness may be removed by filtering the solution through a 0.2 µm filter.
5. Tissues stained with SIGMAFAST™ DAB Substrate Solution may be dehydrated with alcohol and mounted with traditional resinous mounting media.

Troubleshooting

Background is too high

1. Use a blocking step prior to the application of the primary antibody. Diluted normal serum (10% v/v) from the same species as the secondary antibody generally produces the best results.
2. Block endogenous peroxidase by flooding the slide with a solution of 4 parts methanol and 1 part 3% H₂O₂ solution.
3. Decrease the staining time.
4. Titer the conjugate to optimize 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 or peroxidase anti-peroxidase.
5. Increase the staining time.

6. Determine if enzymatic treatment (unmasking) of the antigen is required prior to application of the primary antibody.

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