



Product Information

SIGMAFAST™ 3,3'-Diaminobenzidine tablets

Catalog Number **D4418**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

Synonym: DAB Peroxidase Substrate Tablet Set

Product Description

SIGMAFAST™ 3,3'-Diaminobenzidine (DAB) tablets have been developed for use in immunohistology as a precipitating substrate for the detection of peroxidase activity. 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 coverslipped in the traditional manner and stored for future reference.

$\text{Peroxidase} + 2\text{ H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{ H}_2\text{O}$ (pH 7.6)

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

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 ready-to-use substrate solution containing:

3,3'-Diaminobenzidine (DAB)	0.7 mg/ml
Urea Hydrogen Peroxide (H_2O_2 equivalence, 0.24 mg/ml)	0.67 mg/ml
Tris buffer	60 mM

Components

3,3'-Diaminobenzidine (DAB) tablets Catalog Number D9417	5 or 50 set
Urea Hydrogen Peroxide tablets Catalog Number U1380	5 or 50 set

Reagents and Equipment Required but Not Provided

- Ultrapure water (17 M Ω -cm or equivalent)
- Pipette capable of delivering 15 ml
- Test tubes
- Optional Materials:
 - 0.2 μm filter (Catalog Number F0303)
 - Nickel(II) chloride hexahydrate (NiCl_2 , Catalog Number 223387) or Cobalt(II) chloride hexahydrate (CoCl_2 , Catalog Number 202185) 0.3% (w/v) stock solution for enhancement of tissue stains
 - PBS (Catalog Number P4417) for washing

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Remove the required number of DAB and Urea Hydrogen Peroxide tablets from the freezer. Allow the tablets to reach room temperature. Open DAB tablet package (silver foil) and Urea Hydrogen Peroxide tablet package (gold foil) and drop the tablets into an appropriate container. **Do not touch the tablets with your fingers.** Add 15 ml of ultrapure water. Vortex until dissolved. The SIGMAFAST DAB Substrate Solution is now ready for use. For best results, the solution should be used within one hour.

Storage/Stability

Store the tablets at $-20\text{ }^{\circ}\text{C}$.

Procedure

1. Cover the treated tissue section with 0.2 to 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 NiCl_2 or CoCl_2 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

A. 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.

B. 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.

References

1. Nakane, P.K., and Pierce, G.B., J. Histochem. Cytochem., **14**, 929 (1967).
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5. Merchenthaler, F., *et al.*, Techniques in Immunocytochemistry, **4**, 218 (1989).
6. Hsu, S.M., and Sobane, E., J. Histochem. Cytochem., **3**, 1079 (1982).

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