

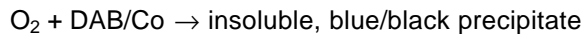
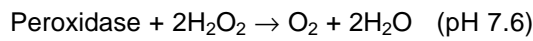
Product Information

SIGMAFAST[™] DAB With Metal Enhancer Tablet Set

Product Number **D 0426**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

Product Description

The SIGMAFAST[™] DAB (3,3'-Diaminobenzidine tetrahydrochloride) with Metal Enhancer Tablet Sets have been developed for use in immunohistochemistry and dot blotting as a precipitating substrate for the localization of peroxidase activity. The DAB reaction has been enhanced by the addition of cobalt chloride. A distinctive, intense, dark blue to bluish black stain is produced that is stable and resistant to alcohol. SIGMAFAST DAB with Metal Enhancer Tablet Sets require no additional ingredients or procedures to prepare an active substrate solution. One DAB/Cobalt tablet and one buffer/urea hydrogen peroxide tablet, when dissolved in 5 ml of ultrapure water, produce 5 ml of ready-to-use substrate solution.



Each SIGMAFAST DAB with Metal Enhancer Tablet Set produces the following solution when dissolved in 5 ml of H₂O:

DAB	0.5 mg/ml
Cobalt Chloride	0.2 mg/ml
Urea Hydrogen Peroxide	0.3 mg/ml
Tris Buffer	0.05 M
Sodium Chloride	0.15 M

Components

SIGMAFAST DAB/Cobalt Tablets (Product No. D 8552)	50
Urea Hydrogen Peroxide Tablets (Product No. U 4756)	50

Reagents and Equipment Required but Not Provided

Ultrapure water
Pipette capable of delivering 5 ml
Test Tubes
Phosphate Buffered Saline (PBS), pH 7.4
(Product No. P 3813) **or**
Tris Buffered Saline (TBS), pH 8.0
(Product No. T 6664)

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/Cobalt (Product No. D 8552) and Urea Hydrogen Peroxide (Product No. U 4756) Tablets from the freezer. Allow the tablets to reach room temperature. Open the DAB/Cobalt tablet package (silver foil) and the Buffer/ Urea Hydrogen Peroxide tablet package (gold foil) and drop the tablets into an appropriate container. **Do not touch the tablets with your fingers.** Add 5 ml of ultrapure water. Vortex until dissolved. The SIGMAFAST DAB with Metal Enhancer Substrate Solution is now ready for use. For best results the solution should be used immediately.

Storage/Stability

Store the tablets at -20°C .

Procedure

1. Cover the tissue section with 0.2 to 0.5 ml of the SIGMAFAST DAB with Metal Enhancer 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, PBS, or TBS.
3. Tissues stained with the SIGMAFAST DAB with Metal Enhancer Substrate Solution may be dehydrated with alcohol and mounted with traditional resinous mounting media.

Note: When finished, dispose of any remaining Substrate Solution in a proper manner.

Troubleshooting Guide

A. Background is too high

1. Use a blocking step prior to the application of 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% H_2O_2 .
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 staining time.
6. Determine if enzymatic treatment (unmasking) of the antigen is required prior to application of the primary antibody.

References

1. Nakane, P., and Pierce, G., J. Histochem. Cytochem., **14**, 929 (1967).
2. Trojanowski, J., *et al.*, J. Histochem. Cytochem., **31**, 1217 (1983).
3. DeJong, A., *et al.*, Histochem. J., **17**, 1119 (1985).
4. Chu, N., *et al.*, J. Histochem. Cytochem., **37**, 257 (1989).
5. Merchenthaler, F., *et al.*, Techniques in Immunocytochem., **4**, 218 (1989).
6. Hsu, S., and Sobane, E., J. Histochem. Cytochem., **30**, 1079 (1982).

RBG,MAM 04/05-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.