Product Information

SIGMA*FAST*™ DAB with Metal Enhancer

Tablet

D0426

Product Description

Diaminobenzidine (DAB) is used in many applications to visualize peroxidase activity.[1-6] The SIGMA*FAST*™ DAB 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.

SIGMA*FAST*™ 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, together produce 5 mL of ready-to-use substrate solution.

Peroxidase + 2 $H_2O_2 \rightarrow O_2 + 2\ H_2O$ (pH 7.6)

O_2 + DAB/Co → insoluble, blue/black precipitate

Each SIGMA*FAST*™ DAB with Metal Enhancer Tablet Set produces the following solution when dissolved in 5 mL of H_2O:

- 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

This product has been used to study such systems as microglia slice cultures,[7] mouse model studies of disease[8,9] and of development,[10] cultured rat lung,[11] porcine molar teeth,[12] and embryos from zebrafish[13] and from *Haliotis* asinina.[14] Several theses[15-18] and dissertations[19-32] have cited use of product D0426 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

DAB/Cobalt Tablets (Component Number D8552): 5 tablets (for 5SET) or 50 tablets (for 50SET)

Urea Hydrogen Peroxide Tablets (Component Number U4756): 5 tablets (for 5SET) or 50 tablets (for 50SET)

Reagents and Equipment Required but Not Provided

- Ultrapure water
- Pipette capable of delivering 5 mL
- Test Tubes
- Phosphate Buffered Saline (PBS), pH 7.4 (such as Cat. No. P3813), or:
- Tris-Buffered Saline (TBS), pH 8.0 (such as Cat. No. T6664)

Preparation Instructions

1. Remove the required number of DAB/Cobalt Tablets (Component Number D8552) and Urea Hydrogen Peroxide (Component Number U4756) Tablets from the freezer.
2. Allow the tablets to reach room temperature.
3. Open the DAB/Cobalt tablet package (silver foil) and the Buffer/Urea Hydrogen Peroxide tablet package (gold foil). Drop the tablets into an appropriate container. **Do not touch the tablets with your fingers.**
4. Add 5 mL of ultrapure water. Vortex until dissolved.

The SIGMA*FAST*™ DAB with Metal Enhancer Substrate Solution is now ready for use. For best results, the solution should be used immediately.

Procedure

1. Cover the tissue section with 0.2 to 0.5 mL of the SIGMA*FAST*™ 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 SIGMA*FAST*™ 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

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% H_2O_2 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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