

op-Metal Enhanced DAB Substrate Kit

Product Description

Sensitive DAB based substrate for chromogenic staining in Immunostaining and blotting technics

Product number: 679921, kit for 300ml final working substrate
contains: 12ml DAB Substrate (J)
6ml Peroxide Substrate (K)
6ml Metal Enhancer (K)
6ml Buffer Stock Solution (K)

Storage: Store DAB Stock Solution at -20°C . Store other reagents at $2-8^{\circ}\text{C}$.
Ship with blue pack (possible with dry ice) (J)

Introduction

3,3'-Diaminobenzidine (DAB) is the most commonly used substrate for demonstrating the presence of horseradish peroxidase in immunohistochemistry or immunoblotting. It produces a stable water/alcohol insoluble reddish brown product. This effect is enhanced by modifying the DAB solution with the addition of metal ions to produce a more dense reaction forming a localized deep blue/black precipitate.^{1,2} Our Metal enhanced DAB Substrate Kit is a stable solution developed to give enhanced color reaction with low background. The reagents supplied are sufficient for making 300ml working solution.

Directions for Use

This a recommended standard protocole. The enhancement with metal is optionnal. Conditions and operating should be modified for specific applications.

Working Solution

1. In 5ml distilled water add 100ul (6 ml) (2 drops) of buffer stock solution.
 2. Add 200ul (12ml) (4 drops) of DAB stock solution.
 3. Add 100ul (6ml) (2 drops) of Hydrogen Peroxide solution. Mix well.
 4. If metal enhancement is desired, add 100ul (6ml) (2 drops) of Metal Enhancing Solution. Mix well.
- This may be used within 6 hours of preparation.

Method FOR TISSUE SECTIONS

1. Add the working solution of DAB and incubate at room temperature for 15 seconds or until color develops.
2. Wash off excess DAB under running water for 5 mins.
3. Counterstain and mount in aqueous or dry medium.

FOR IMMUNOBLOTTING

1. Add the working solution of DAB and incubate at room temperature for 15 seconds or until color develops.
2. Remove excess DAB with two washes in distilled water for 10 mins.
3. Air dry and store in a dark place.

FOR DOUBLE STAINING

Note: It is important to stain the tissue for the first antigen with the metal enhancement.

1. Process the tissue section for labeling the first antigen with peroxidase.
2. Stain with DAB Substrate Kit with metal enhancement.
3. Wash well in distilled water for 5 minutes.
4. Process the tissue section for labeling second antigen with peroxidase.
5. Stain with DAB Substrate kit without metal enhancement.
6. Wash off excess DAB under running water.
7. Counterstain and mount in aqueous or dry medium.

DAB without enhancement will produce a reddish brown precipitate and the Metal Enhancing solution will produce a dark blue/black colored precipitate.

Scientific and Technical Information

Literature

1. (1981) *J. Histochem. Cytochem.* Vol.29, No.6:775
2. (1989) *J. Clin. Pathol.*, **42**:875-880

Warnings

For use *in vitro* only, not for diagnostic.

As DAB and nickel are suspected carcinogens care should be taken in handling and disposal of the working solution. Gloves, lab coats and good laboratory procedures are necessary when using the reagents. For disposal the working solution is discarded in 3% potassium permanganate (KMNO₄) and 2% sodium carbonate in distilled water. Wash out all containers used for substrate staining with this solution. Dispose according to local regulations.

For any information, please contact Uptima, or your local distributor.

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