

eBioscience™ DAB Advanced Chromogenic Kit

Catalog Number: 8801-4965

Also known as: 3, 3'-Diaminobenzidine (DAB) Substrate Solution

RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: eBioscience™ DAB Advanced Chromogenic Kit

REF **Catalog Number:** 8801-4965

Handling Conditions: Reagent A (DAB Chromogen) is a suspected carcinogen, avoid contact with skin and eyes and dispose in accordance with local regulations.

Reagent B (DAB Diluent) contains hydrogen peroxide solution, avoid contact with skin and eyes.



Temperature Limitation: Store at 2-8°C.

Batch Code: Refer to vial

Use By: Refer to vial

Description

This DAB Advanced Chromogenic Kit contains the necessary reagents to prepare a DAB substrate working solution. The enzyme peroxidase (Horseradish Peroxidase/HRP) utilizes the DAB substrate (3, 3'-diaminobenzidine tetrahydrochloride) producing a brownish-red precipitate. These precipitates are insoluble in organic solvent. This enzyme/substrate reaction can be used on tissue sections or cells (grown on culture slides or cytopins) to visualize the localization of an HRP-conjugated antibody in immunocytochemistry and immunohistochemistry applications.

Components

Reagent A (DAB Chromogen) (cat. 00-4966-34): 250 µL; store at 2-8°C.

Reagent B (DAB Diluent) (cat. 00-4967-23): 6 mL; store at 2-8°C.

Labeled Empty Dropper Bottle (cat. 00-4968-60): for storage and application of working DAB solution

Applications Reported

This DAB Advanced Chromogenic Kit has been reported for use in western blotting, microscopy, immunohistochemical staining, and immunocytochemistry.

Applications Tested

This DAB Advanced Chromogenic Kit has been tested by immunohistochemistry of formalin-fixed paraffin embedded human tissue with and without antigen retrieval.

Related Products

00-4953 eBioscience™ IHC /ICC Blocking Buffer - Low Protein

00-4954 eBioscience™ 20X TBS Wash Buffer for IHC/ICC

00-4955 eBioscience™ IHC Antigen Retrieval Solution – Low pH (10X)

00-4956 eBioscience™ IHC Antigen Retrieval Solution – High pH (10X)

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3, 3'-Diaminobenzidine (DAB) Substrate Solution

Introduction

This DAB Substrate Solution contains the necessary reagents to prepare a DAB substrate working solution. The enzyme peroxidase (Horseradish Peroxidase/HRP) utilizes the DAB substrate (3, 3'-diaminobenzidine tetrahydrochloride) producing a brownish-red precipitate. This enzyme/substrate reaction can be used on tissue sections or cells (grown on culture slides or cytopins) to visualize the localization of an HRP-conjugated antibody in immunocytochemistry and immunohistochemistry applications.

General Protocol:

Materials Needed

- Reagent A: DAB Chromogen, 250 uL; store at 2-8°C
- Reagent B: DAB Diluent, 6 mL; store at 2-8°C
- Empty Dropper Bottle: for storage and application of working DAB solution

Handling: Reagent A (DAB Chromogen) is a suspected carcinogen. Avoid contact with skin and eyes and dispose in accordance with local regulations. Reagent B (DAB Diluent) contains hydrogen peroxide. Avoid contact with skin and eyes.

Other Materials Needed

- PBS without sodium azide
- Hematoxylin (optional)
- Ethanol (70%, 90%, 100%) (optional)
- HistoClear II or equivalent (optional)
- Permount Mounting Medium (optional)

Experimental Procedure

Preparation of Working DAB Substrate Solution

1. Add 20 uL of Reagent A (DAB Chromogen) to 500 uL Reagent B (DAB Diluent) in dropper bottle (enough for approximately 5 slides depending on tissue size).
2. Mix carefully but minimize foaming, which will result in poor tissue coverage when applied.
3. Use working solution within two weeks after preparation.
4. Store at 2-8°C protected from light.

Staining Procedure

1. Following incubation with a peroxidase (HRP) detection antibody, wash slides in PBS without sodium azide. The presence of sodium azide will inhibit the enzymatic activity.
2. Apply the working DAB solution using the dropper bottle.
3. Cover the tissue section completely with solution and incubate at room temperature until desired color intensity develops (up to 20 minutes). For best results, monitor slides during development process using a brightfield microscope.
4. Wash the slides well with water.
5. Counterstain with hematoxylin, if desired.
6. Dehydrate in graded series of alcohol and clear with HistoClear II.
7. Mount slides with Permount.

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 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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23 January 2017

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