~~MOSS~~ Mos

DABB 50 1/2

3,3'DIAMINOBENZIDINE CONCENTRATE

DAB

PRODUCT NO. DABM(50X)

For the Localization of Horseradish Peroxidase Labeled Probe May also be Employed for *in situ* Hybridization and Blotting Procedures

INTRODUCTION

3,3' Diaminobenzidine (DAB) is the most common reagent employed for the immunohistochemical detection of horseradish peroxidase (HRP) labeled probes. DAB powder from different sources may show great variability with respect to purity. Histochemical grade material should be pale tan with no purple hue. The purple material is a DAB oxidation product that binds avidly to heme proteins such as myoglobin, hemoglobin and particularly, catalase. It should be obvious that the purple product should be avoided for histochemical uses. **MOSS, INC.** provides a stable DAB concentrate manufactured from carefully selected reagents. When diluted in appropriate buffers with added hydrogen peroxide, the resulting solution provides excellent results with most immunohistochemical and blotting procedures.

METHOD SYNOPSIS

In the presence of HRP and hydrogen peroxide, DAB is oxidized to an insoluble brown polymer easily recognized by light microscopy. The polymer is insoluble in most organic solvents allowing the use of xylene based mounting medium. Similar color is obtained on blotting matrices.

REAGENT PROVIDED

DIAMINOBENZIDINE CONCENTRATE: Contains DAB, 0.0694 Mol L^{-1} in proprietary stabilizers.

Store in refrigerator at 2-8 °C.

Discard if a precipitate forms or if the reagent is purple.

NOTE: DAB is a suspected carcinogen. Wear appropriate protective clothing when handling DAB. Dispose of unused material by pouring into a bleach solution containing 500 mL of commercial hypochlorite containing bleach and 1500 mL of water. Allow to stand several weeks for complete oxidation to occur. Contact local authorities for regulations for discarding the oxidized DAB.

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REAGENT REQUIRED BUT NOT PROVIDED

A. A 0.1 Mol L^{-1} Tris buffer prepared as follows: Dissolve 12.1 g of tris base in approximately 800 mL of reagent grade water. Adjust pH to 7.6 with 2- 6 Mol L^{-1} HCl and QS to 1 L with reagent grade water.

B. Hydrogen peroxide, 0.5 %, prepared from 3 % hydrogen peroxide as follows: Dilute 1.67 mL of 3 % hydrogen peroxide to 10 mL with reagent grade water.

C. Tris/Maleic acid buffer for blotting procedures prepared as follows: Dissolve 12.1 g of tris base in approximately 800 mL of reagent grade water. Adjust pH to 6.0 with a 2 Mol L^{-1} solution of maleic acid. QS to 1 L with reagent grade water.

PROCEDURE-IMMUNOHISTOCHEMICAL

1. To 50 parts of Tris/HCl buffer, add 1 part of DAB Concentrate and 1 part of 0.5 % hydrogen peroxide. Mix well and protect from light.
2. Completely cover tissue sections with this solution and incubate 5-15 minutes at room temperature.
3. After reaction is completed, wash sections thoroughly in reagent grade water.
4. Counterstain with hematoxylin if desired.
5. Dehydrate in graded alcohols to xylene or xylene substitutes.
6. Mount using xylene based mounting media.

PROCEDURE-BLOTTING

1. To 50 parts of Tris/maleic acid buffer, add 1 part of DAB Concentrate and 1 part of 0.5 % hydrogen peroxide. Mix well and protect from light.
2. Completely cover membranes with this solution and incubate 5-30 minutes at room temperature.
3. After reaction is complete, wash membranes thoroughly in reagent quality water.
4. Air dry and store protected from light.

RESULTS

Sites of enzyme activity on tissue sections and blots will appear as brown-orange deposits.