人と科学のステキな未来へ
コスモ・バイオ株式会社

3,3'5,5'-TETRAMETHYLBENZIDINE
TMB
PRODUCT NO. TMBM

A Single Component Precipitating Substrate for the Localization of Horseradish Peroxidase Labeled Probes on Western, Northern, Southern and Dot Blots

INTRODUCTION

3,3'5,5'-Tetramethylbenzidine (TMB), when reacted with horseradish peroxidase (HRP) and hydrogen peroxide, forms a blue free radical cation 1-electron oxidation product. It is routinely used in ELISA procedures. For TMB to be worthwhile as a blotting reagent, enhancers such as dextran sulfate are usually added. Lot-to- lot variations of molecular weights and degree of sulfonation of charged polysaccharides increase the complexity of manufacturing processes. **MOSS, INC.** has developed proprietary methodology that eliminates the use of sugar polymers and guarantees the production of a consistent single component precipitating TMB solution.

METHOD SYNOPSIS

After electropherograms have been transferred to appropriate membranes and reacted with HRP labeled probes, addition of TMBM Solution results in the formation of aquamarine bands at the sites of HRP activity. Similarly, after addition of TMBM Solution to suitably treated dot blots, aquamarine dots appear at the site of enzyme activity.

REAGENT PROVIDED

TMBM SOLUTION: Contains TMB, 1.13 mMol L^{-1}, and hydrogen peroxide, 1.91 mMol L^{-1} and less than 1 % dimethylsulfoxide in a 0.08 Mol L^{-1} acetate buffer, pH 4.9. Also contains non-toxic proprietary stabilizers.

Store at room temperature (15-28 ^{o}C). Storage in refrigerator (2-8 ^{o}C) will not inhibit product performance. Warm to assay temperature prior to use.

Avoid exposure to direct sunlight.

Discard if solution becomes blue or turbid.

PROCEDURE

1. After the final binding reaction with a HRP labeled probe, wash membranes thoroughly with phosphate buffered saline or tris buffered saline containing 0.1 % Tween-20.

NOTE: Do not use reagents containing thimerosal or phenolic compounds as preservatives. Strange phenomenum will be observed if these compounds are present.

2. Following the final wash, cover the membranes with TMBM Solution and incubate with gentle rocking for 5-30 minutes. Longer incubations may be necessary depending upon enzyme activity.

NOTE: If the color develops immediately, the TMB precipitate may flake off the membrane. If this occurs, further dilution of the HRP probe is recommended. A fine blue line circumscribing the band or dot also suggests dilution of enzyme probe to be necessary.

3. Stop reaction by washing thoroughly in reagent grade water. DO NOT WASH WITH BUFFER SOLUTIONS OR TAP WATER. These will cause fading. DO NOT USE ACIDS TO STOP THE REACTION. Acids will turn the bands yellow. Excessive background staining indicates incomplete removal of non-bound HRP from membranes. Increase the wash steps or washing time to remedy this problem.

4. Store dried membranes protected from light.

RESULTS

Aquamarine bands or dots will appear at the site(s) of enzyme activity.

MOSS DOCUMENT TMBM, MARCH 1996 FOR TECHNICAL ASSISTANCE OR OTHER INFORMATION CONCERNING PRODUCTS AVAILABLE FROM MOSS, INC.,
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