3,3',5,5'-TETRAMETHYLBENZIDINE

TMB

PRODUCT NO. TMBH

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

INTRODUCTION

Tetramethylbenzidine (TMB) is an excellent substrate for detecting horseradish peroxidase (HRP) labeled probes. TMB reagents for ELISA and blotting techniques have been available for several years. Histochemically, TMB has been utilized primarily for the study of retrograde axonal transport. To prevent needle formation during color development, sodium nitroprusside in combination with a low pH was used. Recent publications have described stabilization of the TMB precipitate with ammonium hexamolybdate or sodium tungstate. Methyl salicylate has also been employed to render the TMB reaction product insoluble in alcohols. **MOSS, INC.** has now developed a stable-single component TMB for histochemical use containing none of these stabilizers and enhancers. The final reaction product is not affected by ethanol, xylene or xylene substitutes. Thus dehydration through alcohols to xylene and mounting the sections in a xylene type medium is possible. The product may also be used for blotting procedures.

METHOD SYNOPSIS

After reacting with a HRP labeled probe, tissue sections or blots are incubated in the TMB solution. A one electron oxidation occurs resulting in the formation of a stable blue precipitate at the sites of HRP activity.

REAGENTS PROVIDED

Tetramethylbenzidine Solution: Contains TMB, 1.25 mMol L^{-1} in an acetate buffer, pH 4.9. Also contains proprietary stabilizers and precipitating agents.

Store at room temperature

Discard if solution is turbid or blue

Avoid exposure to direct sunlight, iron and copper salts and bleach

Neutral Red Solution: Contains Neutral Red, 0.025% buffered at pH 5.1

Store at room temperature

Discard if a precipitate forms

PROCEDURE

1. Add primary antibody and incubate sections (blots) for an appropriate interval.

2. Wash sections in a buffer such as PBS containing 0.05% Tween 20.

3. Block endogenous peroxidase, if necessary, by incubating sections in absolute methanol at room temperature.

4. Wash briefly in PBS.

5. Incubate sections for 30 minutes at room temperature in PBS containing 0.03% hydrogen peroxide.

6. Wash sections in PBS-Tween-20.

7. Add peroxidase labeled probe and incubate for an appropriate interval

NOTE: The HRP probe must be titered. For example, a 1:50 dilution of an HRP conjugate that performs
well for DAB must be diluted 1:800 for use with TMB.

8. Wash sections in PBS-Tween-20.

9. Cover sections with TMB solution and incubate 5-15 minutes.

10. After TMB incubation is complete, stop reaction bu incubating 10 minutes in deionized water.

11. Counterstain 30-60 seconds in Neutral Red Solution.

12. Rinse in deionized water the dehydrate rapidly through alcohols to xylene.

13. Mount sections using a xylene based medium.

RESULTS

Sites of HRP activity will be brilliant blue.

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