TMB substrate solution for Blotting

Introduction

TMB, namely 3,3',5,5'-Tetramethylbenzidine, is a commonly used substrate for horseradish peroxidase. Under the catalysis of horseradish peroxidase (HRP) or other appropriate peroxidases, TMB produces a blue oxidation product.

This product utilizes HRP to catalyze TMB color development and is intended for use in immunohistochemistry (IHC), in situ hybridization (ISH), or membrane-based color development experiments. This chromogenic solution can also be used for in situ detection of endogenous peroxidase activity in cells or tissues. Upon reaction with HRP-oxidized TMB substrate, the blue reaction product precipitates at the reaction sites and localizes to the membrane surface or tissue samples. When used in Western Blotting, it is recommended to perform stringent blocking and rigorous washing to avoid high background. This product can visualize protein bands in less than one minute. The reaction can be terminated by washing with ddH_2O, and the protein bands remain stable after membrane drying, allowing visualization without special equipment. This product is compatible with both PVDF and nitrocellulose (NC) membranes. For IHC applications, aqueous counterstains and mounting media must be used.

Protocol

1. For Immunohistochemistry (IHC) Detection

   a Perform the experiment according to the standard IHC procedure. After incubation with HRP-conjugated antibody, wash three times with PBS, 10–15 minutes each.

   b After washing, remove the wash buffer and add an appropriate amount of TMB Chromogen Solution.

   c Incubate at room temperature for 30 seconds to 30 minutes (or longer) until the desired color intensity is achieved.

   d Remove the staining solution and add deionized water or double-distilled water (ddH_2O). Incubate for 10 minutes to stop the reaction.

   e Mount the slides and take photographs for documentation.

***Note:** TMB oxidation products are soluble in alcohol and organic solvents. Therefore, use aqueous counterstains and aqueous mounting media.

2. For HRP Color Detection on Membranes

a After incubation with HRP-conjugated antibody according to the corresponding experimental protocol, wash three times with an appropriate washing buffer (PBST/TBST), 10–15 minutes each.

b After washing, remove the washing buffer and add an appropriate amount of TMB Chromogen Solution to completely cover the membrane.

c Incubate at room temperature for 30 seconds to 30 minutes (or longer).

d Photographs can be taken directly, or the reaction can be terminated by washing with ddH_2O before taking photographs.

FAQs

1. Q: High Background Staining

   A: (1) If the background staining is too high, consider using an appropriate blocking buffer (e.g., select a suitable blocking buffer or use 10% serum from the same species as the primary antibody). Also, select a properly adsorbed secondary antibody to reduce non-specific adsorption. (2) Consider shortening the development time or reducing the concentration of the secondary antibody. In addition, using a washing buffer with appropriate stringency or extending the washing time may also help.

2. Q: No Staining or Weak Staining

   A: (1) Appropriately increase the concentration of the primary or secondary antibody. Test the secondary antibody by adding a drop of diluted secondary antibody into a microcentrifuge tube to check whether it can be properly developed. (2) Consider using a more sensitive amplification detection system, such as a biotin-based detection system. (3) Appropriately extend the development time. (4) If the above improvements do not achieve the expected results: for IHC or Western Blot, consider replacing the primary antibody with a more effective one; for Southern, Northern, or in situ hybridization, consider replacing the probe.

Note

1. Store at 4°C, protected from light. Stable for one year.
2. This product is for scientific use only.

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