MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

# TMB Chromogen Solution

**Product Number: BC-TMB-100ml**

## Shipping and Storage

Store at 4 ℃, protected from light. Valid for 1 year.

## Component

| Component | BC-TMB-100ml |
|---|---|
| TMB Chromogen Solution | 100mL |

## Description

TMB Chromogen Solution (for IHC or membrane-based HRP color development) (also referred to as TMB Substrate Solution or TMB Solution for Immunohistochemistry or Blotting) adopts state-of-the-art single-solution TMB chromogenic technology. It develops color under catalysis by horseradish peroxidase (HRP), and serves as a chromogenic substrate for immunohistochemistry, in-situ hybridization or membrane blotting assays. This chromogen solution can also be used for in-situ detection of endogenous peroxidase in cells or tissues.

Conventional TMB chromogenic reagents consist of multiple components which require preparation prior to use. They are prone to precipitate, are relatively inconvenient to handle, and may yield inconsistent results. This TMB Chromogen Solution utilizes the latest TMB chromogenic technology. All relevant reagents are formulated into one single working solution, which simplifies operating procedures and delivers more stable and reliable assay results.

TMB, namely 3,3′,5,5′-Tetramethylbenzidine, is a widely-used substrate for horseradish peroxidase. Upon catalysis by horseradish peroxidase or other suitable peroxidases, TMB generates soluble blue-colored products. The absorbance of the blue product can be typically measured at 370 nm or within the wavelength range of 620-650nm.

## Application

This kit is mainly suitable for HRP-mediated chromogenic detection in immunohistochemistry or in-situ hybridization, as well as membrane-based HRP color development for Western, Southern and Northern blotting assays.

For immunohistochemical detection, 1000 samples can be assayed when the chromogen solution consumption is 0.1mL per sample. For membrane color-development assays, 40 independent detections can be performed with a working volume of 2.5mL chromogen solution per run.

## Protocol

**1. For immunohistochemical detection:**

1.1. Follow standard immunohistochemical procedures. After incubation with horseradish-peroxidase-conjugated antibody, wash the samples 3-5 times with appropriate wash buffer, 3-5 minutes per wash.

1.2. Upon completion of washing, remove the wash buffer and add approximately 100μL TMB chromogen solution drop-wise.

1.3. Incubate at room temperature protected from light for 3-30 minutes or longer (color development may proceed overnight in a humidified chamber) until the desired staining intensity is achieved.

1.4. Discard the staining solution, and incubate samples in deionized water or double-distilled water for 10 minutes to terminate the reaction.

1.5. Capture images, or mount sections appropriately prior to imaging. If necessary, perform counter-staining with Neutral Red Staining Solution, then capture images or mount sections appropriately before image acquisition.

**2. For membrane-based HRP chromogenic detection:**

2.1. Follow the corresponding experimental procedures. After incubation with horseradish-peroxidase-conjugated antibody, wash the membrane 3-5 times with appropriate wash buffer, approximately 5 minutes per wash.

2.2. Remove the wash buffer upon completion of washing.

2.3. Add an adequate volume of TMB chromogen solution drop-wise to fully cover the membrane.

2.4. Incubate at room temperature protected from light for 3-30 minutes or longer (up to several hours) until the desired staining intensity is achieved.

2.5. Capture images directly.

## FAQ

**1. Excessively high background staining**

1.1. If excessively high background staining occurs, on one hand, ensure blocking with an appropriate blocking solution. Options include purchasing a suitable commercial blocking solution or performing blocking with 10 % serum derived from the same host species as the primary antibody. On the other hand, select appropriately-adsorbed secondary antibodies to reduce non-specific binding of the secondary antibody.

1.2. Shortening the color-development time or reducing the secondary-antibody concentration may be considered. In addition, selecting wash buffer of appropriate stringency or prolonging the washing duration will also help mitigate this issue.

**2. No color development or excessively weak staining**

2.1. Appropriately increase the concentration of primary or secondary antibody. Verify the performance of the secondary antibody: add one drop of diluted secondary antibody into a centrifuge tube and test whether the secondary antibody can produce normal color development.

2.2. A more sensitive signal-amplification detection system, such as a biotin-based detection system, may be adopted.

2.3. Prolong the color-development time appropriately.

2.4. If the above improvements fail to yield the expected results, switch to a higher-performance primary antibody for immunohistochemistry or Western blotting. For Southern blotting, Northern blotting or in-situ hybridization, consider replacing the probe.

## Note

1. TMB is irritating to human body. Exercise caution during handling and take appropriate protective measures to avoid direct skin contact or inhalation.
2. This product is a colorless to faint-blue transparent solution. Discontinue use if the TMB horseradish peroxidase chromogen solution turns turbid or develops an intense blue color.
3. This product is for research use only by qualified laboratory personnel. It shall not be used for clinical diagnosis or therapy, food or pharmaceutical applications, and shall not be stored in residential premises.
4. For your personal safety and health, wear a lab coat and disposable gloves while performing operations.