TMB substrate solution for ELISA

Introduction

TMB, namely 3,3',5,5'-Tetramethylbenzidine, is a commonly used substrate for horseradish peroxidase (HRP). Under the catalysis of HRP or other appropriate peroxidases, TMB produces a soluble blue product. After adding a stop solution, the reaction can be measured using a microplate reader. When H_2SO_4 or HCl is used as the stop solution, a yellow product is generated, allowing accurate intensity measurement at 450 nm using a microplate reader. When NaF is used as the stop solution, it exhibits strong light absorption at 650 nm, and the absorbance can be measured between 620–650 nm.

This product utilizes HRP to catalyze TMB color development and is intended for use in ELISA and related experiments. This chromogenic solution can also be used to detect peroxidase levels in samples such as blood or hemoglobin.

Protocol

1. For ELISA Detection

   a Follow the experimental procedure of the ELISA kit. After incubation with HRP-conjugated antibody or streptavidin, wash 3–5 times with an appropriate washing buffer. After the final wash, tap the plate dry on absorbent paper.

   b Add 100 μL of TMB Chromogen Solution to each well.

   c Incubate at room temperature, protected from light, for 10–30 minutes or longer, until the desired color intensity is achieved.

   d To stop the reaction using 2 M H_2SO_4, add 50 μL of 2 M H_2SO_4, then measure the absorbance at 450 nm. If using a lower concentration of acid as the stop solution (e.g., 0.16 M H_2SO_4), it is recommended to add 100 μL. Alternatively, other stop solutions may be used, and the corresponding wavelength should be selected for detection.

2. For Other Suitable Assays Performed in 96-Well Plates (e.g., detection of endogenous peroxidase in tissue or cell samples)

   a Add 10–20 μL of sample directly to each well of a 96-well plate.

   b Add 100 μL of TMB Chromogen Solution.

   c Incubate at room temperature, protected from light, for 10–30 minutes or longer, until the desired color

intensity is achieved.

d Add a stop solution to terminate the reaction and measure using a microplate reader.

FAQs

1. Q: High Background Staining

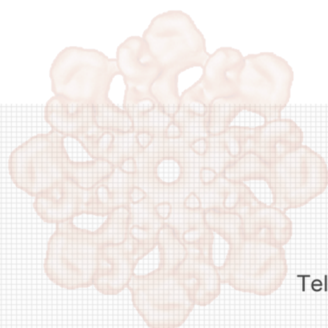
   A: (1) If the background staining is too high, consider using an appropriate blocking buffer (e.g., select a suitable blocking buffer or use 10% serum from the same species as the primary antibody). Also, select a properly adsorbed secondary antibody to reduce non-specific adsorption. (2) Consider shortening 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, consider replacing the primary antibody or the ELISA kit with a more effective one.

Note

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



APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com