Cat. No. 306T

ELISee®

TMB Substrate Kit

Quick Facts

1. Storage conditions before opening: **4 ℃, protected from light for one year.**
2. **50** µL TMB and **50** µL H_2O_2 solution for each well of 96-well plate。
3. Incubate 30-60 mins. Read the blue color at 620-650 nm for kinetic assay.
4. Add **100 µL** stop solution (2N H_2SO_4).
5. Read the yellow color at 450 nm.

Introduction

This ELISee® TMB Substrate kit was designed for horse-radish peroxidase (HRP) detection. As **Fig.1**, the 3,3',5,5'-tetramethylbenzidine (TMB) substrate provides high signal and high sensitivity for ELISA, ELISPOT, IHC and Western blotting. When using a spectrophotometer or a plate reader, the TMB can be catalyzed with peroxidase to produce a blue-color which can be read at 620-650 nm. By addition of 2 N H_2SO_4 stop solution, the blue-color turn into yellow-color, enabling accurate measurement of intensity at 450 nm. When using for blotting, TMB substrate localize the blue-color on the membrane surfaces or tissue samples at sites where the oxidative reaction occurs.

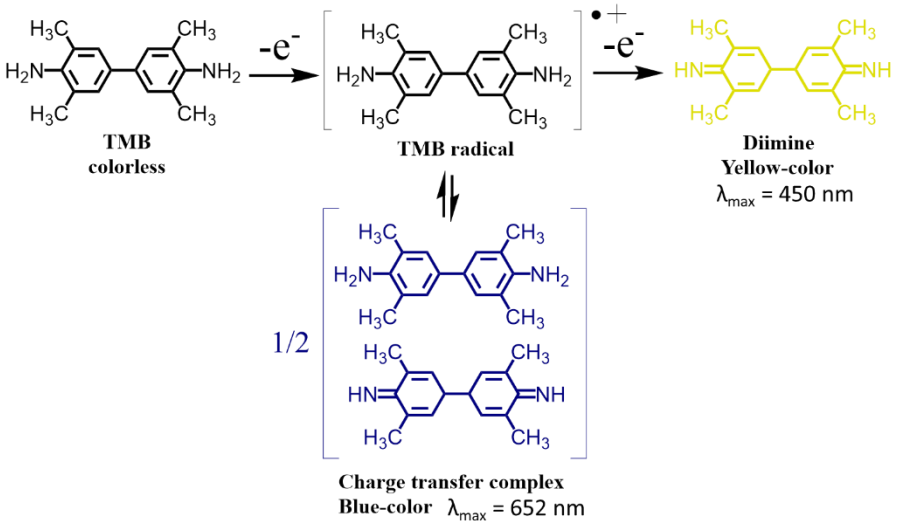

Fig. 1 The principle of TMB substrate.

Kit Contents, Shipping and Storage Information

Contents	1000 tests	Shipping	Storage
TMB solution (Reagent A)	50 mL in brown plastic bottle	Room temperature with protection from light	4℃ with protection from light for one year.
H_2O_2 solution (Reagent B)	50 mL in white plastic bottle		
Stop solution	100 mL in white plastic bottle		

Protocol

1. 96-well plate reader：

1.1 Warm the assay kit to room temperature.

1.2 Transfer 50 µL of TMB solution and 50 µL of H_2O_2 solution to each well.
Note: It is suggested to mix the required volume at a 1:1 ratio, and then divided 100 µL into each well.

1.3 Incubate at room temperature for 30 to 60 minutes.

1.4 Add 100 µL of stop solution (2 N sulfuric acid) to each well.

1.5 Measure absorbance at 450 nm.

2. Western blotting：

2.1 Warm the assay kit to room temperature.

2.2 Remove blot from the transfer apparatus and block nonspecific sites with Blocking Buffer for 10-30 minutes at room temperature with shaking.

2.3 Add the primary antibody and incubate membrane for 1 hour with shaking.

2.4 Wash the membrane with PBS-T.

2.5 Add the HRP-conjugated secondary antibody and incubate membrane for 1 hour at room temperature with shaking.

2.6 Wash membrane with PBS-T.

2.7 According to the required volume, mix the TMB and H_2O_2 solutions of this product in a 1:1 ratio.

2.8 Add the mixed solution to the membrane and carefully monitor color development. Stop the reaction by rinsing membrane with water.

3. Immunohistochemistry：

3.1 Warm the assay kit to room temperature.

3.2 After the incubation of HRP-conjugated secondary antibody, rinse slide three times with PBS.

3.3 According to the required volume, mix the TMB and H_2O_2 solutions of this product in a 1:1 ratio.

3.4 Add the above mixture to cover the tissue section, and carefully monitor the color development while shaking on a shaker.

3.5 To stop the reaction, wash section for 5 minutes with water.

Note: The precipitate is soluble in alcohol and organic solvents, therefore, use an aqueous counterstain and mounting medium.

Product performance

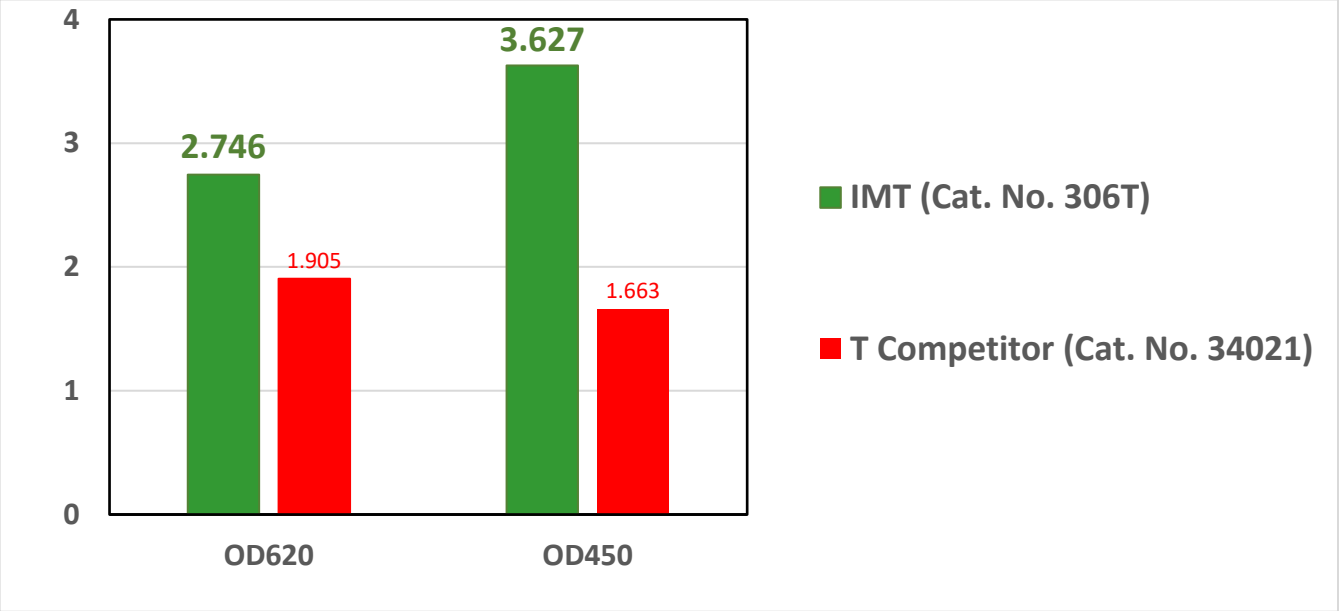

Fig 2. The testing results of this product with other competitor. Using 10 µL of HRP solution (12.5 ng/mL) mix with 50 µL of TMB and 50 µL of H_2O_2 solution, and incubated at room temperature for 45 minutes, measuring OD620. Subsequently, 100 µL of stop solution was added, and OD450 was measured.

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