TMB Substrate Solution

(Cat# C8010; HRP-based ELISA or Blotting reagents; store kit at 4℃)

Introduction

ABS_Bio™ TMB substrate solution is a one-step working solution for HRP (horse-radish peroxidase) detection. TMB substrate solution provides high signal and high sensitivity for ELISA, ELISPOT, IHC and Western blotting. The substrate can be catalyzed with peroxidase to produce a pale blue color which can be read spectrophotometrically at 370 or 620-650 nm. For kinetic assays, read the blue color at 620 - 650 nm. When using for blotting, TMB-Solution is formulated to precipitate and localize the blue reaction product onto membrane surfaces or tissue samples at sites where the peroxidase-substrate reaction occurs. Addition of sulfuric acid stop solution changes the color to yellow, enabling accurate measurement of the intensity at 450 nm using a spectrophotometer or plate reader.

Kit Components

Hydrogen Peroxide Solution:	50 mL	TMB Substrate:	1.5 mL
Stop solution:	50 mL		

Storage and Handling: Store all of the components at 4℃. Shelf Life: 12 months after receipt.

Protocol

96-wells plate ELISA

1. Prepare enough Peroxide Substrate solution by mix 2.5 µL TMB solution with 100 µL Hydrogen Peroxide solution for each reaction well. Transfer 100 µL of Peroxide Substrate solution to each well.
2. Incubate at room temperature for 10 to 15 minutes.
3. Add 100 µL of stop solution (0.16M sulfuric acid) to each well.
4. Measure absorbance at 450 nm.

Western blotting

5. Remove blot from the transfer apparatus and block nonspecific sites with Blocking Buffer for 10-30 minutes at room temperature with shaking.
6. Add the primary antibody and incubate membrane for 1 hour with shaking.
7. Wash the membrane with PBS-T.
8. Add the HRP-conjugated secondary antibody and incubate membrane for 1 hour at room temperature with shaking.
9. Wash membrane with PBS-T.
10. Prepare enough Peroxide Substrate solution by mix 250 µL TMB solution with 10 mL Hydrogen Peroxide solution for 10x10 cm membrane. Add the Peroxide Substrate solution to the membrane and carefully monitor color development. Stop the reaction by rinsing membrane with water.

Immunohistochemistry

After the HRP-conjugated secondary antibody incubation, rinse slide three times with PBS. Add Peroxide Substrate solution to cover the tissue and incubate until significant color develops. 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.

Related Products:

ELISA Coating Buffer(5x, 25mL; #C8001)
ELISA Blocking Buffer(5x 25mL; #C8002)
ELISA Washing Buffer(20x, 25mL; #C8003)
Antibody Dilution Buffer(#C8004)
Protein Loading Buffer(2x, 6x ; #C8005)
SDS-PAGE Running Buffer(10x ; #C8006)
Phosphate Buffered Saline (10x PBS, #C8007)
Phosphate Buffered Saline with Tween 20(10x PBS-T; #C8008)
TBST Buffer(#C8024)
TBST Blocking Buffer(#C8025)