

TMB Substrate



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

rev. 11/14/18

For Research Use Only. Not For Use In Diagnostic Procedures.

Description: TMB Substrate is ready to use for ELISA detection. Reaction between the substrate and immobilized horseradish peroxidase (HRP) conjugated secondary antibodies in the ELISA wells produces a blue colored solution. Color intensity and development time will vary depending on assay sensitivity and conditions. After reaching the desired color intensity, the reaction is terminated by addition of an acidic STOP solution which changes the solution color from blue to yellow. While the results will remain stable for one hour following termination, the plate should be analyzed promptly on a microwell reader at 450 nm. TMB is light sensitive and is therefore packaged in amber bottles to protect the solution from direct sunlight. Recommended storage temperature is 4°C.

Background: TMB (3,3', 5,5'-tetramethylbenzidine) is used as a substrate to generate detectable signal in ELISA. The reaction between the TMB substrate and a peroxidase, typically horseradish peroxidase (HRP), produces a measurable color change that correlates with analyte level. TMB is intended for use in combination with CST's FastScan™ Sandwich ELISA Kits, PathScan® Sandwich ELISA Kits and PathScan® Sandwich ELISA Antibody Pairs.

Directions for Use:

- (a) After incubation with horseradish peroxidase (HRP) conjugated secondary antibody, wash plates with PBS/0.05% Tween-20.
- (b) Add 100 µl of TMB solution/well. Incubate for specific amount of time or until desired signal is achieved. See product datasheet for recommended incubation times for specific antibody pair.
- (c) Add 100 µl of STOP solution.
- (d) Read Absorbance at 450 nm.

Solutions and Reagents: TMB is a one component system that is ready to use from the bottle.

Reagents not supplied: STOP Solution #7002 for stopping peroxidase reaction is not supplied.

Storage: Store at 4°C.

Companion Products:

STOP Solution #7002
Phosphate Buffered Saline (PBS-20X) #9808
Phosphate Buffered Saline with Tween -20 (PBST-20X) #9809
Anti-rabbit IgG, HRP-linked Antibody #7074
Anti-mouse IgG, HRP-linked Antibody #7076
Cell Lysis Buffer (10X) #9803
BSA #9998