

## TMB ELISA Substrate

Cat. No. HE101

Storage: at 2-8°C in dark for one year

### Description

TMB ELISA Substrate is a ready-to-use chromogenic substrate for detection of horseradish peroxidase (HRP) activity. HRP can catalyze 3,3',5,5'-tetramethylbenzidine (TMB) to yield a blue color, the maximal absorbance is at 370 nm or 620-652 nm; however, upon addition of the stop solution, the solution turns to yellow and can be measured at 450 nm

### Kit Contents

Component	HE101-01
TMB Solution	100 ml

### Procedures

1. For ELISA experiment, after incubation with HRP-conjugated secondary antibody, wash ELISA plate 3 times with PBST (PBS containing 0.05% Tween-20), 5 minutes each time.
2. Add 100 µl of TMB Solution (prewarmed to room temperature) to each well. Incubate for 3-30 minutes or longer time in dark until the color of the solution has changed to the expected color.
3. (optional) Add 100 µl of stop solution to each well (prepared by user, 0.5 M H<sub>2</sub>SO<sub>4</sub> or 1 M HCl).
4. Measure the absorbance at 370 nm or 620-650 nm (the reaction without stop solution), or measure the absorbance at 450 nm (the reaction with stop solution).

### Note

If shorter reaction time is needed, incubate the solution at 37°C incubator in dark after adding TMB Solution.

FOR RESEARCH USE ONLY