

## Super TMB ELISA Substrate

Cat. No. HE111

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

### Description

Super 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. Upon addition of the stop solution, the solution turns to yellow and can be measured at 450 nm. This one-component method is 40-50% more sensitive than the traditional TMB ELISA method.

### Kit Contents

Component	HE111-01
Super 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  $\mu$ l 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  $\mu$ l 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 Super TMB Solution.

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