# Soluble TMB Substrate Solution

High sensitivity, non-toxic reagent and convenient to use

www.tiangen.com/en

# Soluble TMB Substrate Solution

**Cat. no. 4995130/4995131**

## Kit contents

| Cat.No. | Product name | Packaging |
|---|---|---|
| 4995130 | Soluble TMB Substrate Solution | 100 ml |
| 4995131 | | 5x100 ml |

## Storage conditions:

Store at 2-8°C away from light with a shelf life of one year.

## Product introduction:

This product provides all the substrate solutions required in ELISA experiments, ready to use in the bottle, easy to operate. The substrate solution contains TMB, $H_2O_2$ and special stabilizers. 3,3', 5,5'-Tetramethylbenzidine (TMB) is a novel substrate for horseradish peroxidase (HRP). The maximum light absorption of TMB is at 285 nm before oxidation and at 370 nm and 652 nm after oxidation. It is recommended to measure the blue light absorption at 652 nm. Addition of acid will change the blue color to yellow, which can be measured at 450 nm, and the sensitivity of detection at 450 nm is higher than that at 652 nm. It is recommended to add 100 µl of termination solution after the substrate oxidation reaction to change the blue color to yellow and then measure at 450 nm.

## Main components

1. TMB
2. Stabilized $H_2O_2$
3. Specialized compounds with the function of accelerating the TMB reaction

## Instructions for use

Please prepare your own termination solution: 1-2 M $H_2SO_4$ before ELISA.

1. Add 100 μl or 150 μl of substrate solution directly into each well of the plate.
2. Incubate at room temperature for 5-30 min.
3. Add 100 μl or 150 μl of termination solution to each well.
4. Place the plate in an enzyme labeling instrument and read the data at 450 nm.

## Common problems

1. Some wells show blue color while others show blue-green color during the substrate reaction.

   This is a normal phenomenon during the TMB substrate reaction and does not affect the results. This is due to the high concentration of horseradish peroxidase (HRP), so it is recommended to reduce the concentration of secondary antibody.

2. Precipitation occurs in wells with over concentration of HRP.

   It is recommended that the concentration of the secondary antibody-HRP be reduced or that 0.5 M $H_2SO_4$ and 8.5 M acetic acid be used as a termination solution. If precipitation occurs after using sulfuric acid as a terminating solution, it is recommended that 100 μl of glacial acetic acid be added to the wells to dissolve the precipitate.

3. If the background reading is high, it is recommended to reduce the concentration of primary or secondary antibody and extend the blocking time.

## Scope of application

- ELISA
- Other applications where HRP is detected by liquid reaction