

NO: CE3001

## HRP Substrate TMB Solutions

### Introduction

TMB solutions are chromogenic reagents for peroxidase, used for ELISA techniques. They are composed of 3,3',5,5'-tetramethylbenzidine (TMB), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and proprietary catalyzing and stabilizing agents. It produces a blue product upon interaction with HRP or HRP conjugates without the addition of hydrogen peroxide. The soluble blue product can be quantitated at 650 nm. Sensitivity is greater than classic substrates like OPD and ABTS, with very low background.

### Storage

2-8°C avoid light for three years. Do not freeze (Return to room temperature before use).

**Seize:** 12mL, 50 mL, 1000mL, 5000mL.

### ELISA Applications

1. Wash the microplate following the incubation of HRP-labeled solution.
2. Add 100 µL TMB Solution into each well. Incubate the plate at 37°C for 15–30 min or until the desired color develops.
3. Measure the absorbance signal at 650 nm with an ELISA microplate reader.  
Note: If desired, the reaction can be stopped by adding an equal volume of 2M sulfuric acid to each well. Stopped reaction should be read at 450 nm.

### NOTE:

1. TMB substrates are optimized for direct and indirect ELISA techniques. They are not suitable for Immunohistochemistry or Western Blotting.
2. Recommendations for use: TMB is sensitive to certain conditions of storage and operating.
3. Exposure to light : TMB substrate is light sensitive. It should be stored in amber vials of HDPE type.
4. Material : the substrate is very sensitive to metallic ions. Only high quality plastic or glass should be used.
5. Avoid the use of caps with rubber joins : this should impair the results.
6. Don't pipet the TMB directly from the bottles : fill a suitable container first with the necessary solution volume to avoid contamination.
7. Don't leave the bottle open too long , and keep it a dark at 2-8°C. Avoid important or frequent variations of temp.