TMB, Soluble
Cat. No. 613544

Description:

TMB is the most sensitive chromogenic substrate for detection of horseradish peroxidase (HRP)-labeled probes. In the presence of HRP and hydrogen peroxide (H_2O_2), TMB is oxidized first to a blue, cation free radical having an absorption maximum at 653 nm (ε = 3.9 x 10^4 $M^{-1}cm^{-1}$). Upon further reaction with HRP/H_2O_2, or addition of acid, the radical is converted to a terminal oxidation product, a yellow diimine that absorbs light at 450 nm (ε = 5.9 x 10^4 $M^{-1}cm^{-1}$). Increased sensitivity (2 - 4 fold) is afforded by the yellow diimine, as its molar extinction coefficient is greater than that of the blue radical.

This TMB substrate is a safe, single-component system for use in HRP-based ELISA. It is optimized with respect to TMB and H_2O_2 concentrations and yields a linear response with the concentrations of HRP usually employed in immunologic assays. Upon reaction with HRP and H_2O_2, the reagent yields a blue soluble end product that is measured at 650 nm. The color formation as a function of time can be recorded or the reaction can be stopped with sodium fluoride for endpoint determinations. Increased sensitivity can be achieved by stopping the reaction with acid, which converts the blue radical to the yellow diimine that is measured at 450 nm.

Physical State:	Liquid, single component system
Storage:	+4°C
Preservatives:	None
Color and Clarity:	Clear to very light blue
pH:	3.1 ± 0.5
Molecular Weight:	240.3
Stability at 4°C:	Passes
Stability at 18-26°C:	Passes
Application:	Check performed on final product in ELISA.
Toxicity:	MSDS available upon request.
Special Instructions:	Protect from exposure to direct sunlight. Discard if the solution turns blue or turbid. The reagent is stable at room temperature, but we recommend storage in the refrigerator for longer shelf life. Warm to assay temperature before use.

Suggested Procedure for Use of TMB in HRP-based ELISAs:

1. Complete all required incubations with antibodies and HRP-labeled probes.
2. Wash plate wells at least 4 times with phosphate-buffered saline (PBS) or Tris-buffered saline (TBS), containing 0.1% TWEEN® 20.
3. After the final wash, shake and blot all residual buffer from the wells.
4. Add 100 µl of TMB solution to each well and incubate for 5 - 30 minutes.
5. Options for measurements:
   a. For kinetic assays, the reaction can be monitored as a function of time by reading absorbance at 650 nm at intermediate intervals.
   b. For endpoint assays that preserve the blue chromogen, the reaction should be stopped by addition of 100 µl of 0.1% sodium fluoride (NaF) and the absorbance read at 650 nm.
   c. If increased sensitivity is desired for endpoint assays, the reaction should be stopped by addition of 100 µl of either 500 mM H_2SO_4 or 250 mM HCl and the absorbance read at 450 nm WITHIN 5 MINUTES. Addition of acid converts the blue radical to the yellow diimine, which absorbs at 450 nm.

NOTE: Optimal incubation times may vary depending on the amount of HRP present. If color develops too quickly, zero-order kinetics will not prevail. Dilution of the probe, antibody, or HRP-labeled reagent may be required. Variations in time, reagent volumes, and temperature may also require further standardization by the user.