BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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TMB ELISA Substrates (Cat #: A-124)

COMPONENTS:	TMB Solution-	120 ml (1200 wells); store at room temperature
	Peroxide Solution-	0.3 ml; store at 4℃
	TMB Stop-	60 ml; store at room temperature

PRODUCT DESCRIPTION: The compound 3,3',5,5'-tetramethylbenzidine (TMB) has emerged as the safest chromogenic substrate of horse radish peroxidase (HRP) exhibiting excellent sensitivity (Tetrahedron 30:3299,1974). HRP in the presence of peroxide converts TMB to a bluish reaction product that can be read spectrophotometrically at 370 or 655 nm. The bluish product upon acidification by HCl or H_2SO_4 generates a bright yellowish color, enabling sensitive detection and quantification by absorbance at 450 nm. This application has become widely used in enzyme-linked immunosorbent assay (ELISA). The substrates solutions are stable for at least one year if stored and handled properly.

PROTOCOLS

Each well of a 96-well ELISA plate requires 0.1 ml working solution. The working solution is prepared in the dark by adding 2 µl of Peroxide Solution to each ml of TMB Solution immediately before the color development step. Calculate the amount of working solution required for each ELISA according to the ratio.

1. Perform the ELISA protocol according to manufacturer's instructions.
2. Perform the final wash step.
3. Prepare working solution as described above. Aspirate off liquid from wells.
4. Add 0.1 ml of working solution to each well, shield plate from light, and incubate at room temperature for ~30 min with gentle agitation. A blue color should gradually appear in wells during incubation.
5. Stop color reaction by adding 50 µl of TMB Stop to each well. Gently agitate the plate to ensure thorough mixing. The solution should turn yellowish instantly upon acidification.
6. Measure absorbance (O.D.) at 450 nm and 540 (or 570) nm using a plate reader. Subtract readings at 540 nm (or 570 nm) from readings at 450 nm to generate ΔO.D..
7. Plot the standard concentrations (x axis) vs. ΔO.D. (y axis) and generate a trendline equation.
8. Use the equation to calculate sample antigen concentrations (see representative SDF1 standard graph of an SDF1 ELISA Kit below).

NOTE:

- TMB Solution is sensitive to photo-degradation and may turn bluish or precipitate during storage if exposed to light. Store the solution at room temperature.
- TMB Stop contains 1N hydrochloric acid (HCl), which can cause skin burns and eye damage. Avoid skin contact.
- Please contact us or visit the product webpage for SDS information on HCl, TMB, and hydrogen peroxide.