INSTRUCTIONS

1-Step[™] Turbo TMB-ELISA



34022		0473.3
Number	Description	
34022	1-Step Turbo TMB-ELISA, 250mL	

Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

TMB (3,3',5,5' tetramethylbenzidine) is a substrate for horseradish peroxidase (HRP). The Thermo Scientific 1-Step Turbo TMB-ELISA is a complete solution that contains TMB and a stabilized hydrogen peroxide. TMB has an absorption maximum at 285nm before oxidation. After oxidation, the maxima are 370 and 652nm. The blue solution can be measured at 652nm; however, upon addition of the stop solution (i.e., acid) a yellow solution results that can be measured at 450nm and has a higher sensitivity than the measurement of the blue solution.

Procedure

Note: Before beginning the ELISA, place the TMB at room temperature and prepare the Stop Solution containing 1-2M sulfuric acid (H_2SO_4).

- 1. Add 100μ L or 150μ L of 1-Step Turbo TMB-ELISA to each well.
- 2. Incubate for 5-30 minutes at room temperature.
- 3. Add 100µL or 150µL of Stop Solution to each well.
- 4. Measure the absorbance at 450nm.

Troubleshooting

Problem	Possible Cause	Solution
Some wells are blue while others are blue/green during the reaction	A high concentration of HRP will tend to produce a greenish result but will not affect performance	Reduce concentration of the HRP or try a less sensitive substrate, such as 1-Step ABTS (Product No. 37615)
A precipitate forms in the wells during the reaction	Concentration of HRP was too high	Reduce the HRP concentration or use a less sensitive substrate
		Add 100µL of glacial acetic acid to dissolve precipitate or use 8.5M acetic acid with 0.5M sulfuric acid as the stop solution
High background	Concentration of HRP was too high or blocking was insufficient	Try a less sensitive substrate or a different blocking buffer
Low signal	Low amount of target protein	Use a more sensitive substrate, such as TMB Substrate Kit (Product No. 34021) or 1-Step Ultra TMB-ELISA (Product No. 34028)



Related Thermo Scientific Products

15031	ELISA Plates, 96-well (strip, corner-notch), clear, 100/pkg
15082	MicroTube Racked Systems, 10 racks
15036	Sealing Tape for 96-Well plates, 100/pkg
37535	SuperBlock [™] Blocking Buffer in TBS, 1L
37545	SuperBlock Dry Blend (TBS), 5 pouches
28376	BupH TM Tris Buffered Saline Packs, 40 packs
28372	BupH Phosphate Buffered Saline Packs, 40 packs
28382	BupH Carbonate-Bicarbonate Buffer Packs, 40 packs

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