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# Datasheet for TMBE-1000 TMB ELISA Peroxidase Substrate

#### **Overview**

Description:	TMB ELISA Peroxidase Substrate - TMBE-1000
Item No.:	TMBE-1000
Size:	1 L
Applications:	ELISA

#### **Product Details**

Background:	TMB (3,3',5,5'-Tetramethylbenzidine) is a colorimetric substrate for peroxidase (HRP)-based enzyme immunoassays, notably ELISAs, and is known for producing a blue color that changes to yellow upon stopping the reaction with sulfuric acid. The color change is quantifiable at 450 nm. TMB's high sensitivity makes it ideal for detecting low concentrations of analytes.
Synonyms:	3,3',5,5'-Tetramethylbenzidine, TMB ELISA substrate, TMBE Substrate, chromogenic ELISA substrate

#### **Target Details**

Purity/Specificity:	TMB ELISA Peroxidase Substrate specifications: pH: 4.0 +/- 0.2
	Stability @ 18° to 26°C: PASS
	Stability @ 4°C: PASS
	QC Raw Material: PASS
	Absorbance check of final product: PASS
	Performance Data per ELISA: PASS
Relevant Links:	TMBE SDS

## **Application Details**



<b>Tested Applications:</b>	ELISA
Application Note:	TMB ELISA Peroxidase Substrate comes ready to use. No dilutions are required. TMB ELISA Peroxidase Substrate will produce a soluble blue end product read at 370 nm or 655 nm. TMB ELISA Peroxidase Substrate incubation time will vary depending on the assay conditions.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1X

### Formulation

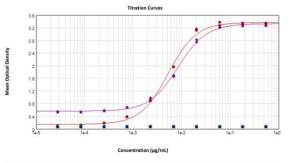
Physical State:	Liquid - clear to very light blue colored liquid
Concentration:	1X
Preservative:	None
Stabilizer:	Proprietary buffer with enhancer

### **Shipping & Handling**

Shipping Condition:	Ambient
Storage Condition:	Store container at 4° C prior to opening. Protect from moisture and light. No special shipping conditions or precautions are required.
Expiration:	Expiration date is one (1) year from date of receipt.

#### Images





<ul> <li>Assay #1</li> </ul>	SARS COV-2 whole spike recombinant protein
Assay #2	SARS COV-2 Spike S1 Subunit recombinant protein
Assay #3	SARS COV-2 Spike S1 (RBD) recombinant protein
· Assay #4	SARS COV-2 Spike Glycoprotein (52) recombinant protein



#### ELISA

ELISA results of Mouse Anti-SARS CoV-2 Spike Protein (S2) Antibody using TMBE-1000. Each well was coated in duplicate with 0.5µg of rec SARS CoV-2 Whole Spike protein [RED line], rec SARS CoV-2 Spike (S1) [GREEN line], rec SARS CoV-2 Spike (S1) RBD protein [BLUE line], and rec SARS CoV-2 Spike (S2) glycoprotein [PURPLE line]. The working dilution for SARS-CoV-2 whole spike is 1:190,000 and SARS CoV-2 Spike (S2) glycoprotein is 1:120,000. The starting dilution of antibody was 0.55µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using Rabbit Anti-Mouse IgG HRP conjugated (p/n 610-403-C46) at 1:8000 and TMB substrate (p/n TMBE-1000). SARS CoV-2 Spike (S2) EC50: 8ng/mL; SARS CoV-2 Whole Spike EC50: 5ng/mL.

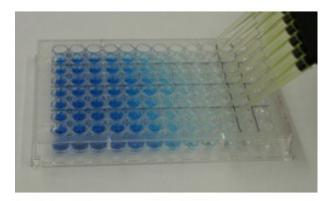
Bottle

TMB ELISA Peroxidase Substrate

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#### ELISA

Rockland Immunochemicals produces a wide variety of buffers and substrates for use in ELISAs. Antigen was diluted in ELISA Microwell Coating Stabilizer (p/n MB-063-0100) added to the microwell plate and incubated overnight at 4°C. The plate was then blocked with ELISA Microwell Blocking Buffer with Stabilizer (p/n MB-064-1000) for 2 hours. The primary antibody was diluted in PBS Fish Gel Concentrate (1:10)(p/n MB-066-0100), added to the plate, and allowed to incubate 1 hour at room temperature. HRP conjugated secondary antibody was diluted in HRP Conjugate Stabilizer (p/n MB-060-0100), added to the plate, and allowed to incubate for 30 minutes at room temperature. TMB ELISA Peroxidase Substrate (p/n TMBE-1000) was added to the plate and allowed to incubate for 30 minutes at room temperature. The reaction was then stopped with 1M HCl and read at 450nm.

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