INSTRUCTIONS

TMB Substrate Kit

34021

0204.3

Number	Description
34021	**TMB Substrate Kit**
	Kit Contents:
	TMB Solution (0.4g/L), 200mL
	Peroxide Solution, 200mL, contains 0.02% hydrogen peroxide in citric acid buffer
	Storage: Upon receipt store kit at 4°C. Kit is shipped at ambient temperature.

Introduction

The Thermo Scientific TMB Substrate Kit contains soluble TMB (3,3´,5,5´-tetramethylbenzidine) that is ideal for sensitive ELISA-based applications. TMB is a chromogenic substrate for horseradish peroxidase. Once oxidized by the enzyme, this substrate yields a blue product that absorbs at 370nm and 652nm. Adding sulfuric or phosphoric acid to stop the reaction changes the product to yellow that absorbs at 450nm.

Procedure for using TMB for ELISAs

Note: Color variations of the TMB Solution are normal and will not interfere with ELISA results.

1. Immediately before use, mix equal volumes of the TMB Solution and the Peroxide Solution.
2. Add 100µL of the TMB substrate solution to each microplate well.
3. Incubate plate at room temperature for 15-30 minutes or until the desired color develops.

   Note: High concentrations of HRP yield a greenish solution. Stop the reaction before any wells display a green product. A precipitated product indicates the presence of too much HRP and the need to optimize experimental conditions.
4. Stop reaction with 100µL of 2M sulfuric acid or 8M acetic acid with 1M sulfuric acid.
5. Measure the absorbance of each well at 450nm.

Related Thermo Scientific Products

15075	**Reagent Reservoirs,** 200/pkg.
15082	**Microtube Racked System,** 10 racks
15036	**Sealing Tape for 96-Well Plates,** 100/pkg.
21130	**Pierce® High Sensitivity Streptavidin-HRP,** 0.5mL
31030	**Pierce High Sensitivity NeutrAvidin®-HRP,** 0.5mL
21124	**Streptavidin, Horseradish Peroxidase Conjugated,** 2mg
31002	**NeutrAvidin, Horseradish Peroxidase Conjugated,** 2mg
37538	**StartingBlock™ (PBS) Blocking Buffer,** 1L
37542	**StartingBlock (TBS) Blocking Buffer,** 1L

General References

Bos, E.E., *et al.* (1981). 3,3'5,5'-Tetramethylbenzidine as an Ames test negative chromogen for horseradish peroxidase in enzyme immunoassay. *J Immunoassay* **2:**187-204.

Josephy, P., *et al.* (1982). The horseradish peroxidase-catalyzed oxidation of 3, 5,3',5' Tetramethylbenzidine. *J Biol Chem* **257(7):**3669-75.

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