ReadiUse™ TMB Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*

Catalog number: 11003, 11012
Unit size: 1 L, 100 ml

Component	Storage	Amount	
		Cat No. 11003	Cat No. 11012
ReadiUse™ TMB Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	Refrigerate (2-4 °C), Minimize light exposure	1 L	100 mL

OVERVIEW

Horseradish peroxidase (HRP) and HRP conjugates facilitate the ABTS oxidation in the presence of hydrogen peroxide, turning ABTS into its blue-green oxidized product. ReadiUse™ TMB Substrate Solution is a premixed solution of TMB substrate with hydrogen peroxide. It produces a blue product upon interaction with HRP or HRP conjugates without the addition of hydrogen peroxide. The soluble blue product can be quantitated at 650 nm. Use of a stop solution enhances sensitivity 2-4 fold and the resulting yellow solution can be read at 450 nm. ReadiUse™ TMB Substrate Solution provides an convenient and ultrasensitive quantitative substrate system.

AT A GLANCE

Important
Warm ReadiUse™ TMB Solution to room temperature before use.

Note The reagent is to be used as supplied, no dilution is required.

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	650 nm
Recommended plate:	Solid white

SAMPLE EXPERIMENTAL PROTOCOL

1. Wash the assay plate following the incubation of HRP-labeled reagent.
2. Add 100 µL of ReadiUse™ TMB Solution into each well.
3. Incubate the plate at room temperature for 15 – 30 min or until the desired color develops.

   Note The incubation time varies depending on the assay conditions.
4. Measure the absorbance signal at 650 nm with an ELISA microplate reader.

   Note If desired, the reaction can be stopped by adding an equal volume of 2M sulfuric acid to each well. Stopped reaction should be read at 450 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate HRP samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

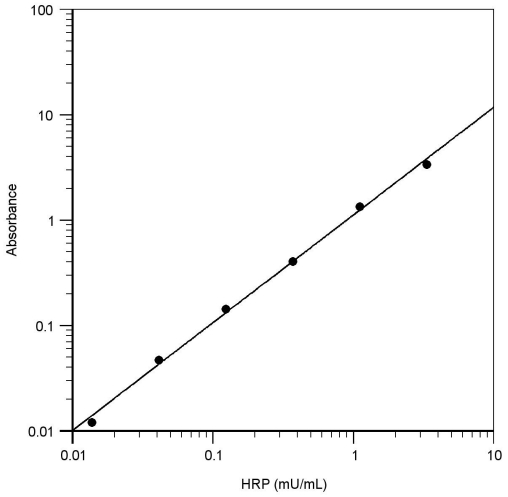

Figure 1. HRP dose response was measured with ReadiUse™ TMB Substrate Solution in a clear 96-well plate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER