

## ReadiUse<sup>TM</sup> TMB Substrate Solution

\*Optimized for ELISA Assays with HRP Conjugates\*

Ordering Information:	Storage Conditions:	Instrument Platform:
Product Number: 11003 (1 L)	Keep at 2 – 8 °C Avoid exposure to light	Colorimetric microplate readers

## Introduction

AAT Bioquest's one step ReadiUse™ TMB Substrate Solution is optimized for ELISA assays that use horseradish peroxidase (HRP)-labeled conjugates and hydrogen peroxide in microwell plates or test tubes. Our TMB solution allows the following HRP reaction kinetics to be readily followed.

$$TMB + H_2O_2 \xrightarrow{HRP} TMB^+ + H_2O$$

$$(\lambda_{max} = 650 \text{ nm})$$

HRP and HRP conjugates facilitate the above reaction in the presence of hydrogen peroxide, turning TMB into its blue oxidized product. The oxidized TMB product has the absorption maximum of 650 nm that can easily be followed with a spectrophotometer. Use of a stop solution enhances sensitivity 2-4 fold and the resulting yellow solution can be read at 450 nm. ReadiUse<sup>TM</sup> TMB Substrate Solution provides an ultrasensitive quantitative substrate system. The product is extremely stable, it is supplied ready to use with no mixing required.

## **ELISA Assay Protocol for one 96-well plate**

- 1. Warm ReadiUse™ TMB Solution to room temperature before use. *Note: The reagent is to be used as supplied, no dilution is required.*
- 2. Wash the assay plate following the incubation of HRP-labeled reagent.
- 3. Add 100 μL of ReadiUse<sup>TM</sup> TMB Solution into each well.
- 4. 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.*
- 5. 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.

**Disclaimer:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact our technical service representative for more information.