



Product Data Sheet

**Ready-to-use 1-Component Liquid TMB Substrate for HRP (ELISA)**  
(3,3',5,5'-tetramethylbenzidine)

Cat# 80091

Sizes: □, 500 ml

Store at 4°C

**DESCRIPTION**

Ready-to-use 1-component liquid TMB develops a deep blue product when reacted with peroxidase (in the form of peroxidase labeled conjugates) in the microwell plates. The expected appearance of the substrate solution is colorless to light amber or light blue. Presence of light bluish color will not affect the performance of the product; in the absence of peroxidase, addition of stopping solution will convert it to a colorless solution.

**FORM/STORAGE/STABILITY**

Ready-to-use 1-component liquid TMB substrate is supplied in two convenient sizes of 100 and 500 ml. Store at 4°C. The substrate solution is stable for one year at 4°C.

**CONTENTS**

Ready-to-use 1-component liquid TMB substrate contains 3,3',5,5'-tetramethylbenzidine in an acidic buffer. The concentration of H<sub>2</sub>O<sub>2</sub> is 0.01%.

**USES**

**Preparation**

No preparation necessary; the solution is ready-to-use. Warm to room temperature before use.

**Suggested Applications**

1-Component liquid TMB substrate is recommended for use in detecting HRP in microwell ELISA. It is not recommended for membrane or immunohistochemical staining assays. (Please consult Alpha Diagnostic International product catalog for appropriate substrates for membrane applications.)

**Recommended Substrate Volume for ELISA**

After thorough plate washings (automated ELISA plate washers are recommended), add 50-200 µl of substrate solution into each well. The volume of substrate solution must not exceed the volume of HRP-conjugate. Depending on the concentration of enzyme, color will develop almost immediately. Tap gently to mix.

**Recommended Handling**

Ready-to-use 1-Component liquid TMB substrate is **incompatible** with silicone based materials.

**ABSORBANCE MEASUREMENTS**

**Kinetic Assays**

1-Component liquid TMB substrate produces a blue color. Measure absorbance at 650 nm.

**Endpoint Assays**

The reaction of substrate and enzyme can be terminated by the

addition of a stopping solution (Cat # 80100) which will change the blue color to a yellow. Measure absorbance at 450 nm.

**Stopping Solution**

Alpha Diagnostic Intl. offers stopping solution (Cat# 80100) for TMB substrate. For the termination of endpoint assays, it is recommended that an equal volume (to that of substrate used) of 0.18 M sulfuric acid be added to the wells. After adding the stop solution, the color can be read at 450 nm. Stopped reactions (yellow color) are stable for several hours if the plate is kept covered and in the dark. However, it is recommended to read the plate within 60 min.

**When to Stop Substrate Reaction**

The point at which the substrate reaction (color development) is stopped is often determined by the ELISA reader being used. The reaction may be stopped before the positive wells reach an O.D. of about 2.0. Note: The addition of stopping solution increases the O.D. values 2-3 fold.

**Substrate Reaction Too Fast?**

In order to reduce the intensity of color development, it is recommended that the enzyme conjugate and/or antibodies be further diluted. **Dilution of the substrate is not recommended.**

**High sensitivity substrate #80092 is also available that provides about 3-10 fold more color as compared to #80091.**

The product is for in vitro research use only.

**MSDS:** available at web site or contact ADI

**Related items available from ADI**

- 80012 High binding ELISA Strips plates (8 wellsx12 strips)
- 80050 ELISA Plate Coating buffer concentrate (10X)
- 80060 ELISA Plate Blocking Buffer concentrate (10X) BSA-based
- 80062 ELISA Plate Blocking Buffer concentrate (10X) milk-based
- 80070 Antibody and Conjugate Diluent for ELISA concentrate (10X)
- 80080 Wash buffer concentrate (20X) for ELISA
- 80081 Phosphate Buffered Saline (PBS, pH 7.4) concentrate (20X)
- 80091 TMB substrate (1-component) for ELISA
- 80092 TMB substrate (1-component) for ELISA (**high sensitivity**)
- 80100 Stop solution for TMB substrate (ELISA) concentrate (10X)
- 80110 Single solution TMB substrate (membrane) solution for western blot
- 80160 Rabbit Serum Antibody detection ELISA kit, Qualitative (mouse, goat, sheep, monkey, human, chicken kits are also available)

80091-TMB-Substrate-Solution

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