Product Data Sheet

Ready-to-use 1-Component Liquid TMB Substrate for HRP (Membrane/Western blot)
(3,3',5,5'-tetramethylbenzidine)

Cat # 80110 **Sizes**: , 100 ml , 500 ml Store at 4oC

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 4^0C. The substrate solution is stable for one year at 4^0C.

CONTENTS

Ready-to-use 1-component liquid TMB substrate contains 3,3',5,5-tetramethylbenzidine in an acidic buffer. The concentration of H_2O_2 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 (Alpha Diagnostic International, Catalog No. 80100) which will change the blue color to a yellow. Measure absorbance at 450 nm.

Stopping Solution

Alpha Diagnostic Intl. offers stopping solution (**catalog no. 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.**

MSDS: Available at the web site or contact ADI.

The product is for in vitro research use only.

Related items available from ADI

Catalog#	ProdDescription
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
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)

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