

TMB One Component HRP Microwell Substrate



Catalog No. **Size**
E102 2 x 60 ml

Product Description:

TMB One Component HRP Microwell Substrate contains 3,3',5,5'-tetramethylbenzidine in a mildly acidic buffer. The substrate is supplied as a ready to use solution. Unreacted substrate should be colorless or very light yellow in appearance. When this substrate system is reacted with peroxidase, a soluble blue reaction product is obtained. The reaction can be stopped using appropriate stop solution, producing a soluble yellow or soluble blue reaction product, depending upon the stop reagent used, which is stable for at least 1 hour. TMB One Component HRP Microwell Substrate is not recommended for membrane or immunohistochemical applications that require a precipitating reaction product.

Storage and Product Stability:

Store at 2°C - 8°C. This substrate is light sensitive and should be protected from direct sunlight and UV sources.

Hazard Identification:

TMB One Component HRP Microwell Substrate is not a hazardous substance or mixture. Please refer to the SDS (www.bethyl.com/product/E102) for additional information.

Preparation:

TMB One Component HRP Microwell Substrate is supplied as a ready to use solution. The product should be allowed to equilibrate to room temperature (25°C) prior to use.

Recommended Substrate Volume:

After thorough plate washing, add 100 µL of substrate solution to each well. Color will begin to develop immediately and come to completion in 15 – 30 minutes.

Absorbance Measurements:

Kinetic Assays:

A soluble blue reaction product develops which can be read at 370 nm or in a range of 620 nm to 650 nm. For best results, sample absorbance values should be monitored and read before absorbance values exceed 2.0 OD units.

Endpoint Assays:

After stopping the TMB substrate becomes yellow. Read at a wavelength of 450 nm.

Stopping Solution: The substrate reaction can be stopped using equal volume (100 µL) of 1 N HCl or 0.18 M H₂SO₄. (ELISA Stop Solution, Cat. No. E115, may be purchased from Bethyl Laboratories.) Addition of acid turns the blue color to yellow and stops the enzymatic reaction.

When to Stop Substrate Reaction? The point at which the substrate reaction is stopped is often determined by the ELISA reader being used. Many ELISA readers record absorbance up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate stopped before positive wells are no longer recordable. Since stopping the reaction increases sample absorbance values approximately three fold, sample OD values should be monitored and substrate reaction stopped when values reach approximately 0.7 OD units.

Substrate Reaction Too Fast? To reduce the intensity of the substrate reaction, it is recommended that the HRP conjugate and/or antibodies in the immunoassay be further diluted. Dilution of the substrate is not recommended.

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