



TMB One Component HRP Microwell Substrate

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.

Hazard Identification:

TMB One Component HRP Microwell Substrate does not contain aprotic solvents. Please refer to the MSDS for additional information.

Product Stability, Storage and Specifications:

TMB One Component HRP Microwell Substrate is stable for a minimum of 48 months from the date of manufacture when stored at 2°C - 8°C. Storage is recommended at 2°C - 8°C. This substrate is light sensitive and should be protected from direct sunlight and UV sources.

Product Use:

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. For microwell applications, 100 µL of substrate solution is added to each well. 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. In endpoint assays, the substrate reaction can be stopped using equal volumes of 1 N HCl, 0.6 N sulfuric acid, or one of the BioFX Stop Solutions for TMB Microwell (Cat. No. STRP, LSTP, BSTP and LBSP). Addition of acid turns the blue color to yellow and stops the enzymatic reaction. In the case of the 650 nm Stop Solutions for TMB Microwell (Cat. No. BSTP and LBSP), the blue color does not change. Since stopping the reaction increases sample absorbance values approximately three fold, unless using the 650 nm Stop Solutions for Microwell, sample OD values should be monitored and substrate reaction stopped when values reach approximately 0.7 OD units. After stopping with the 450 nm Stop Solutions for TMB Microwell (Cat. No. STPR and LSTP), a soluble yellow product develops which is read in the 450 nm range. Stopping with the 650 nm Stop Solutions for TMB Microwell (Cat. No. BSTP and LBSP) produces a soluble blue product which is read in the 650 nm range. Dilution of the substrate is not recommended. To reduce the intensity of a reaction, it is recommended that the antibodies or conjugates be diluted.

References:

1. Walsh, S., A. Shah, and J. Mond. 2003. *Antimicrob. Agents Chemother.* 47:554.
2. Lillehoj, E.P., G.M. Ford, S. Bachmann, J. Schroder, E.F. Torrey, and R.H. Yolken. 2000. *J. Neurovirol.* 6:492.
3. Yang, Y., O. Dukhanina, B. Tang, M. Mamura, J.J. Letterio, J. MacGregor, S.C. Patel, S. Khozin, Z. Liu, J. Green, M.R. Anver, G. Merlino, and L.M. Wakefield. 2002. *J. Clin. Invest.* 109:1607.

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