Leinco Technologies, Inc.
excellence in early discovery research™

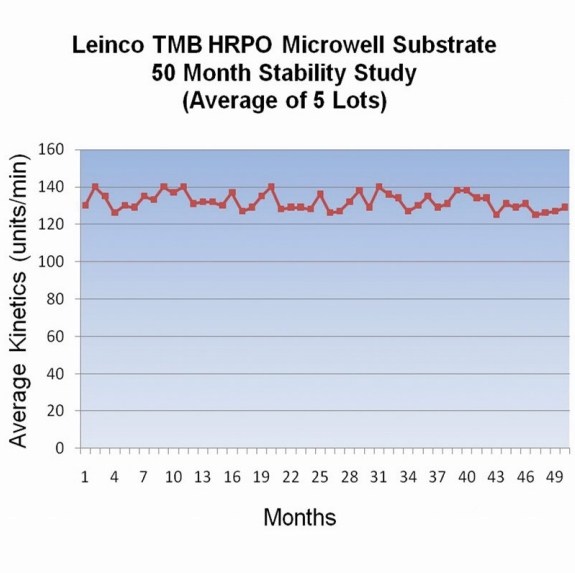
TMB Substrate Solution

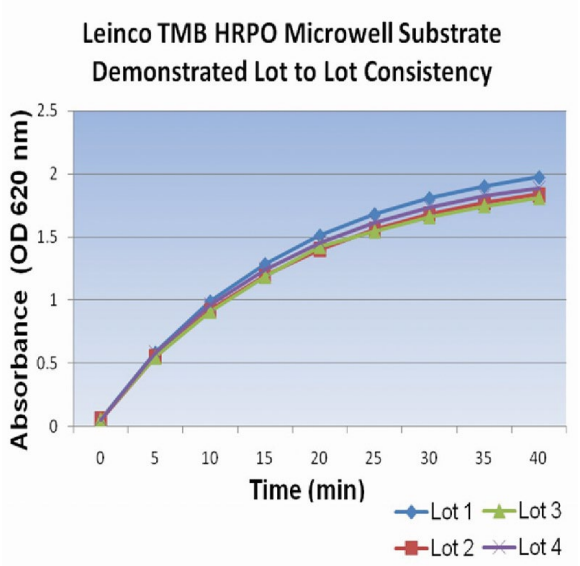
'Ready to Use'

Substrate and Assay Reagents

Product Information

Product No.: T118
Storage: 2° to 8°C





Product Description

Background:

TMB Microwell Substrate (3,3',5,5' tetramethylbenzidine) is a soluble substrate used with the enzyme horseradish peroxidase (HRPO) designed for various qualitative or quantitative immunoassays but not recommended for membrane or immunohistochemical applications where a precipitating reaction product is required. Initially, the substrate should be colorless or slightly yellow in color and will be stored in a mildly acidic buffer. TMB Microwell Substrate turns a deep blue color when oxidized with hydrogen peroxide catalyzed with horseradish peroxidase labeled conjugates with absorbencies at 370 nm or in a range of 620 nm to 650 nm. The color is changed to a bright yellow if an acidic stop reagent such as HCl or sulfuric acid is used. The absorbance should be read at 450 nm if the reaction is stopped which increases the sensitivity 2-4 fold.

Leinco Technologies' TMB Microwell Substrates exhibit superior kinetic performance, sensitivity and lot to lot consistency as compared to other vendors. The outstanding shelf life of at least forty eight months for the TMB Microwell Substrate makes this reagent ideal for long term use of the same manufacturing lot.

Directions for Use

TMB Microwell Substrate is a ready to use solution that needs no preparation or dilution (Note: Allow the substrate to equilibrate at room temperature (~25°C) prior to use). Pour estimated amount of substrate into a suitable high quality plastic reservoir to avoid contamination of the bulk solution. Again, it is recommended that you allow the substrate solution to equilibrate to room temperature before use. While the TMB solution is equilibrating, wash the microplates thoroughly to remove excess peroxidase labeled conjugates. Washing the plates at least four times is recommended to minimize background noise.

410 Axminister Dr, St. Louis, MO 63026 (800) 538-1145 leincoglobal@leinco.com

End-Point Assays:
1.) Add 100 µl of substrate solution to each well of a 96 well ELISA plate.
2.) Allow the substrate reaction to develop and wait for a soluble blue reaction product to develop(Note: This typically takes 15-20 minutes).
3.) The reaction should be stopped by using a 450 nm or 650 nm stop solution. (Note: A soluble yellow product develops after stopping and stabilizing with a 450 nm stop solution.)
4.) Measure the absorbance at 450 nm or 650 nm of each microwell within 1 hour.

Kinetic Assays:
1) Add 100 µL of equilibrated substrate solution to each microtiter plate well.
2) Measure the absorbance at 650 nm at multiple time points.
3) Calculate the rate of absorbance change for each microwell.

Storage and Stability
The high quality of the substrate can be preserved by storing at temperatures between 2° to 8°C. The substrate should not be frozen and should be protected from direct light by storing in amber bottles.

DO NOT FREEZE

Country of Origin
USA