

**Product Datasheet**  
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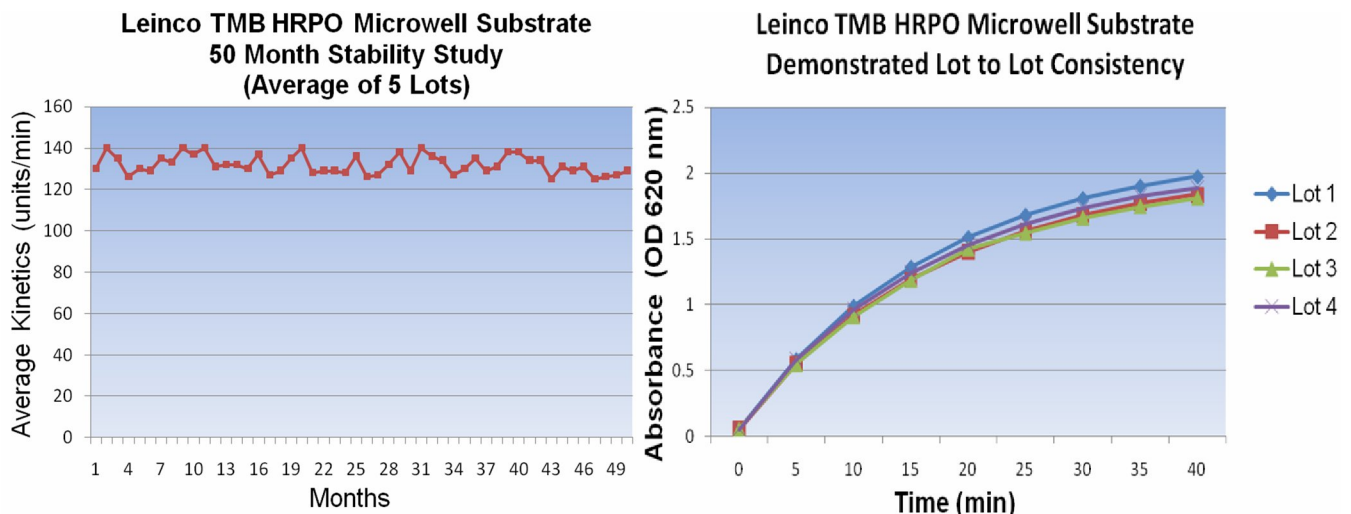
**TMB HRPO Microwell Substrate  
Horseradish Peroxidase Standard Kinetic Substrate  
One Component "Ready Use"**

**Prod. No.:** T118  
**Pkg. Size:** 100 ml, 500 ml, 1L  
**Storage:** 2 – 8°C *Detailed storage instructions below.*

**Description**

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.



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## Directions for Product Use

TMB Microwell Substrate is a ready to use solution that needs no preparation or dilution. Pour estimated amount of substrate into a suitable high quality plastic reservoir to avoid contamination of the bulk solution. 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.

Add 100  $\mu$ l of substrate solution to each well of a 96 well ELISA plate. Once a soluble blue reaction product develops, the plate can be read at 370 nm or in the range of 620 nm to 650 nm. The absorbance values of the sample should be monitored so that a linear curve can be plotted. For increased sensitivity, add 50  $\mu$ l of a stop solution such as 1.0 N HCl or 1.0 N sulfuric acid to create a soluble bright yellow reaction product. After stopping the enzymatic reaction the plate should be read at 450 nm. Estimated incubation times for substrate range from 20 to 30 minutes.

## Storage and Stability

The high quality of the substrate can be preserved by storing at temperatures between 2 – 8°C. When properly stored, TMB Microwell Substrate is stable for a minimum of 48 months from the manufactured date. The substrate should not be frozen and should be protected from direct light by storing in amber bottles. Avoid substrate contact with metals or silica based materials. Only high quality glass and plastic products should be used for storing aliquots.

## Reported Applications

Soluble TMB is suitable for use in sensitive ELISA based assays

## Country of Origin

USA

## Related Products

TMB HRPO Microwell Substrate Slow Kinetic One Component "Ready Use" ([Leinco Prod. No.: T117](#))  
TMB HRPO Microwell Substrate UltraSensitive™ One Component "Ready Use" ([Leinco Prod. No.: T263](#))  
TMB HRPO Microwell Substrate Conductivity One Component "Ready Use" ([Leinco Prod. No.: T271](#))  
UltraAvidin™-HRPO ([Leinco Prod. No.: A106](#))  
Streptavidin-HRPO ([Leinco Prod. No.: S554](#))  
Goat Anti-Mouse IgG (H&L)-HRPO ([Leinco Prod. No.: M114](#))  
Goat Anti-Human IgG (H&L)-HRPO ([Leinco Prod. No.: H603](#))  
Goat Anti-Armenian Hamster IgG (H&L)-HRPO ([Leinco Prod. No.: A128](#))  
Goat Anti-Rabbit IgG (H&L)-HRPO ([Leinco Prod. No.: R1190](#))  
Goat Anti-Rat IgG (H&L)-HRPO ([Leinco Prod. No.: R1215](#))



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## Troubleshooting Guide

Problem	Possible Causes	Possible Solutions
High Background Noise	<ul style="list-style-type: none"> <li>Insufficient plate washing</li> </ul>	<ul style="list-style-type: none"> <li>Increase number of washes</li> <li>Add detergent or protein to wash solution</li> <li>Allow a short soak period between washes</li> </ul>
	<ul style="list-style-type: none"> <li>Concentration of enzyme is too high</li> </ul>	<ul style="list-style-type: none"> <li>Check calculations and titrate if necessary</li> </ul>
	<ul style="list-style-type: none"> <li>High incubation time</li> </ul>	<ul style="list-style-type: none"> <li>Reduce incubation time</li> </ul>
	<ul style="list-style-type: none"> <li>Contaminated buffers or solutions</li> </ul>	<ul style="list-style-type: none"> <li>Repeat assay with fresh buffers and solutions</li> </ul>
No/Low Signal	<ul style="list-style-type: none"> <li>Capture antibody did not bind to plate</li> </ul>	<ul style="list-style-type: none"> <li>Evaluate coating conditions and standardize</li> <li>Increase coating time</li> <li>Increase coating concentration</li> <li>Change plate type to high binding</li> </ul>
	<ul style="list-style-type: none"> <li>Contaminated buffers or incorrect solutions</li> </ul>	<ul style="list-style-type: none"> <li>Repeat assay with fresh buffers and solutions</li> </ul>
	<ul style="list-style-type: none"> <li>Not enough reporter antibody used</li> </ul>	<ul style="list-style-type: none"> <li>Increase concentration of HRPO labeled antibody</li> </ul>
Poor assay-to-assay reproducibility	<ul style="list-style-type: none"> <li>Inconsistent washing</li> </ul>	<ul style="list-style-type: none"> <li>Standardize washing and ensure thoroughness</li> </ul>
	<ul style="list-style-type: none"> <li>Variations in incubation temperature</li> </ul>	<ul style="list-style-type: none"> <li>Ensure constant temperature during incubations</li> <li>Ensure all reagents are at constant temperature when added</li> </ul>
Low reading across entire plate	<ul style="list-style-type: none"> <li>Incorrect wavelength on plate reader</li> </ul>	<ul style="list-style-type: none"> <li>Check filters</li> <li>Check absorbance wavelength (either 650 nm or 450nm)</li> </ul>
	<ul style="list-style-type: none"> <li>Insufficient development time</li> </ul>	<ul style="list-style-type: none"> <li>Increase development time until background appears</li> </ul>

## References

- Josephy, P.D. *et al.* (1982). *J. Biol. Chem.* **257**(7):3669.  
 Mond, J. *et al.* (2003) *Antimicrob. Agents Chemother.* **47**:554.  
 Yolken, R.H. *et al.* (2000) *J. Neurovirol.* **6**:492.  
 Wakefield, L.M. *et al.* (2002) *J. Clin. Invest.* **109**:1607.



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