

ABTS HRPO Microwell Substrate Horseradish Peroxidase Substrate One Component “Ready Use”

Prod. No.: A202
Pkg. Size: 100 ml, 500 ml, 1 L
Storage: 2 – 8°C *Detailed storage instructions below.*

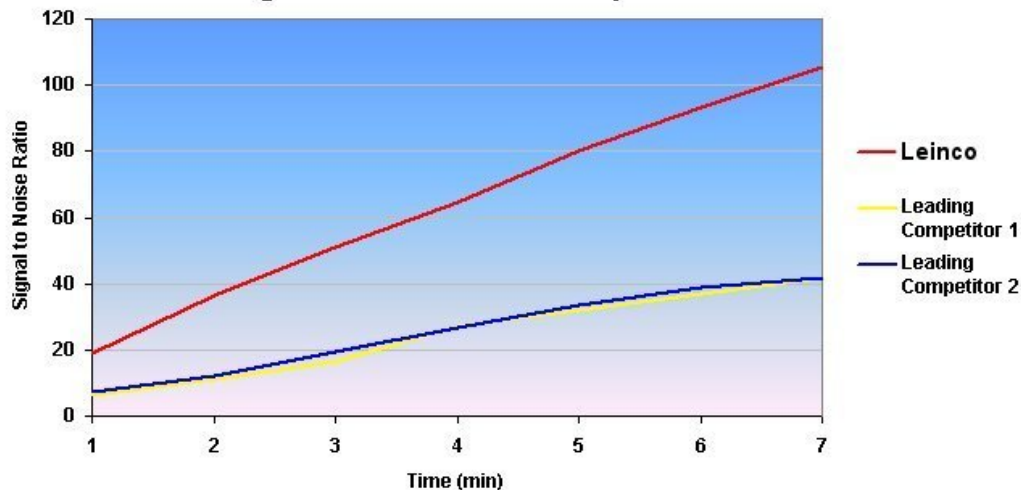
MSDS

Description

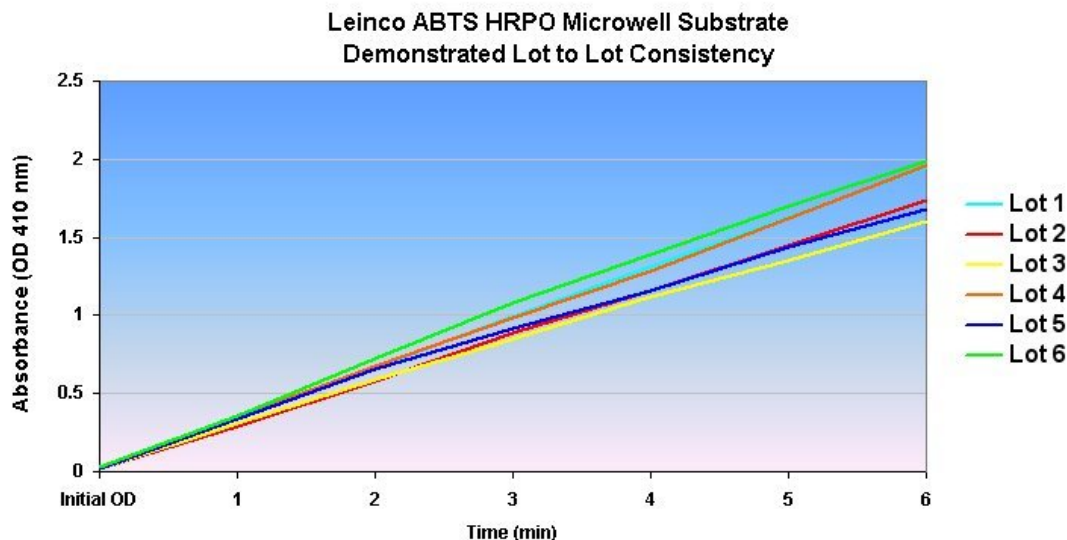
ABTS HRPO Microwell Substrate (2,2'-azino-di[3-ethylbenzthiazoline sulfonate (6)]) is a soluble substrate used with the enzyme horseradish peroxidase (HRPO) designed for various qualitative and quantitative immunoassays but not recommended for membrane or immunohistochemical applications where a precipitating reaction product is required. Initially, the substrate should be colorless or very light green in color and is formulated in a mildly acidic buffer. ABTS HRPO Microwell Substrate turns a blue-green color when reacted with horseradish peroxidase labeled conjugates with absorbencies at 405 nm to 410 nm.

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

Leinco Technologies' ABTS HRPO Microwell Substrate Signal to Noise Ratio Comparison



Products are for research use only. Not for use in diagnostic or therapeutic procedures.



Directions for Product Use

ABTS HRPO 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 ABTS 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 μ l of substrate solution to each well of a 96 well ELISA plate. Once a soluble blue-green reaction product develops, the plate can be read at 405 nm to 410 nm. The absorbance values of the sample should be monitored so that a linear curve can be plotted. The substrate reaction can be stopped by addition of 100 μ l of a stop solution such as 1% sodium dodecyl sulfate (SDS) or 405/410 nm Stop Reagent for ABTS Microwell ([Leinco Prod. No.: A204](#)). OD values should be read at 405 nm to 410 nm with or without the addition of stop solution. Estimated incubation times for substrate range from 15 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, ABTS HRPO Microwell Substrate is stable for a minimum of 18 months from the manufactured date. The substrate should not be frozen and should be protected from direct light by storing in amber bottles. Only high quality amber glass and plastic products should be used for storing aliquots.

Reported Applications

ABTS HRPO Microwell Substrate is suitable for use in sensitive ELISA based assays



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Product Datasheet

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Country of Origin

USA

Related Products

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](#))

405/410 nm Stop Reagent for ABTS Microwell ([Leinco Prod. No.: A204](#))

[Chromogenic Substrates](#)

Troubleshooting Guide

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



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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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