Leinco Technologies, Inc.™
excellence in early discovery research

TMB HRPO Membrane Substrate
One Component "Ready Use"

Prod. No.: **T116**
Pkg. Size: **100 ml, 500 ml, 1 L**
Storage: **2 – 25°C** Detailed storage instructions below.

MSDS

Description

TMB HRPO Membrane Substrate (3,3',5,5' tetramethylbenzidine) is a precipitating substrate used with the enzyme horseradish peroxidase (HRPO) designed for various membrane immunoassays but not recommended for microwell or immunohistochemical applications. Initially, the substrate should be slightly yellow to pink in color and is formulated in a mildly acidic buffer. TMB HRPO Membrane Substrate turns a deep blue color when reacted with horseradish peroxidase labeled conjugates.

Leinco Technologies' TMB HRPO Membrane Substrate is a highly sensitive, high performance formulation that produces a high signal to noise ratio with low membrane background. The outstanding shelf life of at least thirty months for the TMB Membrane Substrate makes this reagent ideal for long term use of the same manufacturing lot.

Western blot showing a serial dilution (10µg to 40 ng) of a 70 kDa protein

Directions for Product Use

TMB HRPO Membrane Substrate is a ready to use solution that needs no preparation or dilution. It is recommended that you allow the substrate solution to equilibrate to room temperature before use. Pour the necessary amount of substrate onto the blocked HRPO conjugate labeled membrane to completely cover the membrane. The substrate will react with sites on the membrane containing peroxidase, producing an insoluble permanent dark blue reaction product. The reaction should be monitored and read before the background color becomes too intense which may result in diminished contrast between the positive and background staining. To stop the reaction, rinse the membrane with reagent quality water. If a less intense reaction is desired, reduce the concentration of the HRPO labeled conjugate. Do not dilute the substrate.

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

Storage and Stability

The high quality of the substrate can be preserved by storing at temperatures from 2 – 25ºC. When properly stored, TMB HRPO Membrane Substrate is stable for a minimum of 30 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

TMB HRPO Membrane Substrate is suitable for use in Western blotting.

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)

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Troubleshooting Guide

Problem	Cause	Solution
Too much background signal observed on membrane	TMB substrate was left on the membrane too long	Decrease the amount of time the TMB substrate is on the membrane
	Too much primary antibody used	Decrease the amount of primary antibody used and wash with TBST for 5 minutes after the primary antibody incubation
	Too much secondary antibody used	Decrease the amount of secondary antibody used
Nonspecific bands show up on the membrane	Too much primary antibody used	Decrease the amount of primary antibody used and wash with TBST for 5 minutes after the primary antibody incubation
	Too much secondary antibody used	Decrease the amount of secondary antibody used
Signal disappears from membrane	Membrane not stored correctly	Store the membrane in the dark in High Purity Water
No signal is observed on the membrane	Low amounts of specific protein present	Expose the membrane to TMB substrate for a longer period of time. Include positive control(s) during analysis
	Insufficient primary antibody used	Use more primary antibody
	Insufficient secondary antibody used	Use more secondary antibody
	Protein degraded into fragments	Add protease inhibitors to original sample before running a gel

References

Polisky, B., *et al.* (2001). *Nat.Biotechnol.* **19**:62.

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