

TMB 1-Component HRP Membrane Substrate

A one-component formulation suitable for membrane assays using HRP.

TMB 1-Component HRP Membrane Substrate is suitable for membrane applications using horseradish peroxidase (HRP) as the conjugated detection enzyme. TMB 1-Component HRP Membrane Substrate should not be used for microwell (ELISA) applications.

TMB 1-Component HRP Membrane Substrate is a one-component formulation containing stabilized 3,3',5,5'-tetramethylbenzidine (TMB). The TMB substrate is oxidized by the peroxidase enzyme to yield an insoluble dark blue reaction product. TMB 1-Component HRP Membrane Substrate is supplied ready-to-use at 1X.

Best results are obtained by equilibrating TMB 1-Component HRP Membrane Substrate to room temperature (25°C) prior to use. After probing with the antibody and HRP reagents, wash membrane thoroughly and transfer the membrane into a clean container. Cover the membrane surface with ample amount of TMB 1-Component HRP Membrane Substrate and incubate 5-20 minutes. The substrate will react with sites on the membrane containing peroxidase, producing an insoluble permanent dark blue reaction product.

For best results, monitor the substrate color development process until the target protein bands are visible. To stop the reaction, rinse the membrane with reagent quality water. If the reaction proceeds too long, there will be excessive background staining and diminished resolution of the target peptide or protein banding regions. If the color development is too rapid or intense, it is recommended to dilute the antibodies or conjugates or shorten the incubation period.

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ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

TMB 1-COMPONENT HRP MEMBRANE SUBSTRATE

Size **Catalog#**
100 mL 6280

INSTRUCTIONS:

1. Perform electro-blotting procedure.
2. Block membranes 4 hours-overnight.
3. Probe membranes with antibodies and HRP conjugate.
4. Wash membranes after each antibody incubation step. Always transfer to a clean container for substrate development step.
5. Bring TMB 1-Component HRP Membrane Substrate to room temperature; protect it from light.
6. Add TMB 1-Component HRP Membrane Substrate to cover the membrane surface.
7. Incubate TMB 1-Component HRP Membrane Substrate at room temperature for 5-20 minutes.
8. Monitor the substrate color development to visualize the target peptide and protein bands.
9. Stop the color development reaction by transferring the membrane to diH₂O. Change the solution several times to ensure complete removal of all soluble TMB components.
10. Analyze the data.

For more ELISA information and protocols, please visit www.immunochemistry.com.

SPECIFICATIONS:

- Faint yellow to pink liquid
- 1X ready to use

STORAGE:

- 2-8°C
- Protect from light

SAFETY & USAGE:

- SDS available at immunochemistry.com
- Not for human or drug use
- For research use only

