V1.1 Revised 07/06/2020

TMB Substrate (Immunoblotting)

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

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

TMB Substrate (Immunoblotting) 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 Substrate (Immunoblotting) is supplied ready-to-use at 1X.

Best results are obtained by equilibrating TMB Substrate (Immunoblotting) 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 Substrate (Immunoblotting) 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.

TMB Substrate (Immunoblotting)

Size	Catalog#
100 mL	LS-M40-100

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 Substrate (Immunoblotting) to room temperature; protect it from light.
6. Add TMB Substrate (Immunoblotting) to cover the membrane surface.
7. Incubate TMB Substrate (Immunoblotting) 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 diH_2O. 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.LSBio.com.

SPECIFICATIONS:

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

STORAGE:

- 2-8°C
- Protect from light

SAFETY & USAGE:

- SDS available upon request
- Not for human or drug use
- For research use only

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