Product Information

3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System for Membranes

Ready-to-use solution

T0565

Product Description

3,3',5,5'-Tetramethylbenzidine (TMB) is a chromogenic substrate suitable for use in colorimetric procedures which utilize horseradish peroxidase (HRP) conjugates.[1-4] These procedures include ELISA and immunoblotting.

This product is supplied as a one-component, ready-to-use HRP substrate that contains TMB in a mildly acidic buffer. It is optimized for use with either nitrocellulose or PVDF Western blotted (transferred and probed) membranes. This solution develops a dark blue, insoluble reaction product in the presence of HRP. Prior to reaction with HRP, the solution is colorless to light yellow. The TMB solution has been optimized to reduce membrane background. Due to the insoluble reaction product, the TMB solution is not compatible with ELISA applications.

This TMB solution has similar sensitivity levels to chemiluminescent detection reagents, as it can detect as little as 0.15 ng. The TMB solution provided is enough for at least 25 mini-gel sized (10 × 10 cm) blots.

Several publications,[5-8] theses,[9] and dissertations[10-16] cite use of this product in their research protocols.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

This procedure assumes that the Western blotting (transfer and probing) steps have already occurred and that HRP-conjugates are bound to the blot.

1. Place the membrane on a clean flat sheet of plastic wrap.
2. Use enough TMB solution to completely cover the membrane's surface. Typically 3 mL is enough to cover a mini-gel sized (10 × 10 cm) membrane.
3. Expose the membrane to the TMB solution at room temperature for 5-15 minutes. Visually monitor the reaction.
4. Remove the substrate when protein bands are visible and the background is still low. High background diminishes the contrast between positive signal and background.
5. Wash the membrane in ultrapure water (such as Cat. No. W4502) for 1 minute.
6. Capture the image of the wet membrane using a camera or scanner.
7. Store the membrane dry and in the dark. If stored correctly, signal should remain on the membrane for a week.

Storage/Stability

The product as supplied is stable for at least 1 year if stored at 2-8 °C. Keep the substrate out of direct sunlight.

References

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Troubleshooting Guide

Below are some common problems and their corresponding solutions associated with Western Blotting detection using TMB substrate.

Problem	Cause	Solution
Too much background signal observed on membrane	TMB substrate was left on the membrane too long	Decrease the amount of time that the TMB substrate is on the membrane.
	Too much primary antibody used	Decrease the amount of primary antibody used. 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. Wash the membrane with TBST for 5 minutes after 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 ultrapure water.
	Signal Degrades over time	Signal will degrade after a week even if membrane is stored in the dark, capture image with a camera or scanner.
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 a positive control(s) during analysis.
	Insufficient primary antibody used	Use more primary antibody.
	Insufficient secondary antibody used	Use more secondary antibody.
	Protein degraded into proteolytic fragments	Add protease inhibitors to original sample before running a gel.

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