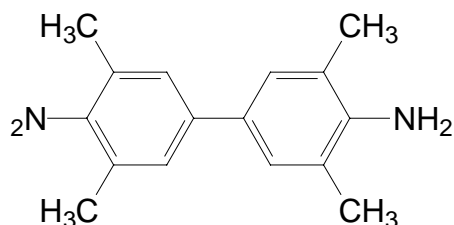


**3,3',5,5' TETRAMETHYLBENZIDINE (TMB)
LIQUID SUBSTRATE SYSTEM FOR MEMBRANES**

Product Number **T 0565**
Store at 2-8°C

Product Information**TECHNICAL BULLETIN****Product Description**

Structure of 3,3',5,5'-tetramethylbenzidine (TMB)



This product is supplied as a ready-to-use horseradish peroxidase (HRP) substrate containing 3,3',5,5'-tetramethylbenzidine (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**.

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

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.

Precautions and Disclaimer

This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Components Needed for a Complete Western transfer and blot

- High Purity Water (W 4502)
- SDS-PAGE gels, Running buffer (T 7777), and gel running apparatus
- Color Marker to be used as a control. For Western blotting it is best to use Chemichrome Western Control (C 4236).*
- Nitrocellulose (N 5891) or PVDF (P 4188) membranes
- Blotting Paper (P 7796)
- Western Transfer Buffer (T 4904)
- Methanol (T1775)
- Western Blotting apparatus
- Blocking Solution, for example Western Blocker Solution (W 0138).*
- Primary monoclonal IgG antibody specific to protein to be detected. For FLAG[™] based systems we recommend Anti-FLAG[™] M2 monoclonal antibody (F 3165).
- Secondary antibody-HRP conjugate specific for the primary antibody system, for example Anti-Mouse IgG HRP conjugate (A 9044).*
- Wash Solution, for example Tris Buffered Saline with 0.05% Tween 20 (TBST, T 9039).*

* Instead of ordering items separately, try ProteoQwest[™] Colorimetric Western Blotting Kit, TMB Substrate (PQ0101). It contains the following:

- Chemichrome Western Control (C 4236)
- Western Blocker Solution (W 0138)
- Wash solution; Tris Buffered Saline with 0.05% Tween 20 (T 9447)
- Anti-Mouse IgG Horse Radish Peroxidase conjugated secondary antibody (A 5225)
- TMB Substrate (T 0565)

Procedure

This product is a one component ready-to-use substrate. This procedure assumes that Western blotting (transfer and probing) steps have already occurred and HRP-conjugates are bound to the blot.

1. Place the membrane on a clean flat sheet of plastic wrap. Use enough TMB solution to completely cover the membrane's surface. Typically 3 ml is enough to cover a mini-gel (10 X 10 cm) sized membrane.
2. Expose the membrane to the TMB solution at room temperature for 5-15 minutes. Visually monitor the

reaction. Remove the substrate when protein bands are visible and the background is still low. High background diminishes the contrast between positive signal and background.

3. Wash the membrane in High Purity Water (W 4502) for 1 minute.
4. Capture the wet membrane's image using a camera or scanner.
5. Store the membrane dry and in the dark. If stored correctly, signal should remain on the membrane for a week.

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 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 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 High Purity 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 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.

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