

# 1-Step Ultra TMB-Blotting Solution

**37574**

2397.0

Number	Description
37574	1-Step Ultra TMB-Blotting Solution, 250mL

**Storage:** Upon receipt store product at 4°C. Product is shipped at ambient temperature.

## Introduction

The Thermo Scientific 1-Step Ultra TMB-Blotting Solution is a ready-to-use, single-component horseradish peroxidase (HRP) substrate for Western blotting applications (not for use in ELISA assays or immunohistochemical applications). The high sensitivity, increased signal-to-noise ratio and low background blotting solution contains soluble TMB (3,3',5,5'-tetramethylbenzidine), which reacts with HRP to produce an insoluble dark blue precipitate.

## Example Procedure for Western Blot Detection

This protocol is a general guideline for using 1-Step Ultra TMB-Blotting Solution in a Western blot. Optimal conditions for each specific system must be empirically determined.

**Note:** Overdevelopment may cause the substrate precipitate to flake off the membrane. Color development rates vary depending on the amount of protein and detecting antibodies present and the blocking solution used. For best results, use an appropriate blocker and carefully monitor color development.

### A. Materials Required

- Wash buffer [e.g., Phosphate-buffered saline with Tween<sup>TM</sup>-20 Detergent (PBS-T): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (Product No. 28372) with 0.05% Tween-20 Detergent **or** Tris-buffered saline with Tween-20 Detergent (TBS-T): 25mM Tris, 0.15M sodium chloride; pH 7.2 (Product No. 28376) with 0.05% Tween-20 Detergent].

**Note:** Use only high-quality Tween-20 Detergent such as Thermo Scientific Surfact-Amps 20 Detergent Solution (Product No. 28320), which is specifically purified to be free of peroxides and carbonyls that may interfere in some systems.

- Blocker (e.g., Thermo Scientific Clear Milk Blocker, Product No. 37587; StartingBlock in PBS, Product No. 37578; StartingBlock in TBS, Product No. 37579)

**Note:** 0.05% Tween-20 Detergent can be added to the formulation to reduce nonspecific binding.

- Antigen-specific primary antibody diluted with Blocker. For best results, empirically determine the optimal dilution for each specific system.
- HRP-conjugated secondary antibody diluted with wash buffer. For best results, empirically determine the optimal dilution for each specific system.

### B. Method

**Note:** Equilibrate the 1-Step Ultra TMB-Blotting Solution to room temperature before use.

- Remove blot from the transfer apparatus and block nonspecific sites with Blocker for 10-60 minutes at room temperature with slight agitation.
- Add the primary antibody and incubate membrane for 60 minutes at room temperature (or overnight at 4°C) with slight agitation.
- Wash the membrane for 10 minutes with PBS-T or TBS-T. Repeat for a total of three washes.

4. Add the HRP-conjugated secondary antibody and incubate membrane for 60 minutes at room temperature with slight agitation.
5. Wash membrane for 5 minutes with PBS-T or TBS-T. Repeat for a total of five washes.
6. Remove all buffer solution and add 1-Step Ultra TMB-Blotting Solution to cover the membrane; carefully monitor color development. Stop the reaction by rinsing membrane at least two times with ultrapure water.

**Note:** The fully colored bands develop within 5-30 minutes, but timing must be optimized for each assay. Development time depends on the amount of antigen and antibody used in the system.

## Troubleshooting

Problem	Possible Cause	Solution
No detection	Low antigen amount was loaded onto the gel	Increase antigen amount
	Insufficient concentration of primary and/or secondary antibody	Optimize primary and secondary antibody dilutions
High background	Development time was too long	Reduce development time
		Increase amount of antigen and/or optimize primary and secondary antibody dilutions
	Insufficient water wash	Add sufficient amount of water and increase the number of water washes
		Air-dry membrane facedown on a flat surface

## Additional Information Available on Our Website

- Tech Tip #24: Optimize antigen and antibody concentrations for Western blots
- Tech Tip #32: Guide to enzyme substrates for Western blotting
- Tech Tip #43: Protein stability and storage

## Related Thermo Scientific Products

28320	Surfact-Amps™ 20 Detergent Solution, 6 × 10mL
88018	Nitrocellulose Membrane, 0.45µm, 33cm × 3m, 1 roll
88518	PVDF Transfer Membrane, 0.45µm, 26.5cm × 3.75m, 1 roll
26681	Pierce™ Prestained Protein Molecular Weight Marker, 1 × 48 microtube plate
37538	StartingBlock™ (PBS) Blocking Buffer, 1L
37542	StartingBlock (TBS) Blocking Buffer, 1L
37528	Blocker™ Casein in PBS, 1L
37530	Blocker BLOTTO in TBS, 1L
37520	Blocker BSA in TBS (10X), 125mL
28372	BupH™ Phosphate Buffered Saline Packs, 40 packs
28376	BupH Tris Buffered Saline Packs, 40 packs

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