INSTRUCTIONS

1-Step™ TMB-Blotting

34018

0148.2

Number	Description
34018	**1-Step TMB-Blotting**, 250mL

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

Introduction

The Thermo Scientific 1-Step TMB-Blotting is a one component horseradish peroxidase (HRP) substrate for Western blotting and immunohistochemistry. TMB is highly sensitive and generates a blue-purple precipitate. This substrate is provided ready to use for maximum convenience.

Example Procedure for Western Blot Detection

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

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 Thermo Scientific SuperBlock Blocking Buffer – Blotting (Product No. 37517) and carefully monitor color development.

A. Materials Required

- Phosphate Buffered Saline with Tween®-20 Detergent (PBS-T): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (Product No. 28372) with 0.05% Tween-20

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

- SuperBlock® Blocking Buffer – Blotting (Product No. 37517) containing 0.05% Tween-20
- Antigen-specific primary antibody diluted with Blocking Buffer. For best results, empirically determine the optimal dilution for each specific system.
- HRP-conjugated secondary antibody diluted with Blocking Buffer. For best results, empirically determine the optimal dilution for each system being tested.

B. Method

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

1. Remove blot from the transfer apparatus and block nonspecific sites with Blocking Buffer for 10-30 minutes at room temperature with shaking.
2. Add the primary antibody and incubate membrane for 1 hour with shaking.
3. Wash the membrane with PBS-T.
4. Add the HRP-conjugated secondary antibody and incubate membrane for 1 hour at room temperature with shaking.
5. Wash membrane with PBS-T.
6. Add the 1-Step TMB-Blotting to the membrane and carefully monitor color development. Stop the reaction by rinsing membrane with water.

Example Procedure for Immunohistochemical Staining

This protocol is a general guideline for using 1-Step TMB-Blotting in an immunohistochemical application. Optimal conditions for each specific system must be determined empirically.

A. Important Procedural Notes

- To minimize potential microbial contamination, carefully handle reagents and use ultrapure water in all solutions. Avoid touching slides and do not allow dust or other debris to contaminate samples, tissues or other material.
- Do not use sodium azide as a preservative for buffers as it inhibits HRP activity.
- Use a humidity chamber set at 20-25°C for all incubations to prevent evaporation. Additionally, completely cover the tissue section with solution during incubations to prevent drying.
- Adjust the standard protocol according to antigen concentrations. High antigen concentrations will require less incubation time to obtain optimal staining. When reducing incubation times, increase incubation temperature to 37°C.
- An ABC complex system, such as the Thermo Scientific ABC Standard Peroxidase Staining Kit (Product No. 32020) or Ultra-Sensitive ABC Standard Peroxidase Staining Kit (Product No. 32050), can increase sensitivity if necessary.

B. Materials Required

- Phosphate Buffered Saline (PBS): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (Product No. 28372) containing 0.05% Tween-20

  Note: Use only high-quality Tween-20 such as Surfact-Amps® 20 Detergent Solution (Product No. 28320), which is a specially purified Tween-20 that is free of peroxides and carbonyls that may interfere in some systems.
- Blocking Buffer: Thermo Scientific StartingBlock (PBS) Blocking Buffer (Product No. 37538) with 0.05% Tween-20 or StartingBlock™ T20 (PBS) Blocking Buffer (Product No. 37539), which is pre-formulated with Tween-20
- Antigen-specific primary antibody diluted with Blocking Buffer. For best results, empirically determine the optimal dilution for each specific tissue/antigen type being tested.
- HRP-conjugated secondary antibody diluted with Blocking Buffer. For best results, empirically determine the optimal dilution for each system being tested.

C. Method

7. Fix cryostat sections in acetone for 10 minutes and allow them to air-dry.

   Note: Paraffin sections must be de-paraffinated with xylene and rehydrated with descending ethanol washes. If picric acid was used during fixation, incubate overnight in PBS followed by several PBS washes.

8. Quench endogenous peroxidase activity by incubating tissue for 30 minutes in 0.3% hydrogen peroxide in methanol. Omit this step if endogenous activity is not a problem or if the antigen will not survive exposure to H_2O_2.

   Note: Use a humidity chamber set at 20-25°C for all incubations to prevent evaporation. Additionally, completely cover the tissue section with solution during incubations to prevent drying.

9. Wash tissue with PBS. Add Blocking Buffer and incubate for 30-60 minutes at room temperature.
10. Apply the primary antibody to the tissue and incubate for 30-90 minutes.
11. Rinse tissue three times for 10 minutes with PBS. Apply the HRP-labeled secondary antibody to the tissue and incubate for 30 minutes.
12. Equilibrate the 1-Step TMB-Blotting to room temperature. For best results, filter the substrate solution using a glass fiber filter paper immediately before use. Do not use hydrophobic membrane filters, which will retain the TMB substrate and reduce sensitivity.
13. Rinse slide three times for 10 minutes with PBS. Apply 1-Step TMB-Blotting solution to the tissue and incubate until significant color develops. To stop the reaction, wash section for 5 minutes with water.

    Note: The precipitate is soluble in alcohol and organic solvents, therefore, use an aqueous counterstain and mounting medium.

Additional Information

Please visit the website for additional information relating to this product including the following items:

- Tech Tip #24: Optimize antigen and antibody concentrations for Western blots
- Tech Tip #16: Block endogenous biotin
- Tech Tip #32: Guide to enzyme substrates for Western blotting
- Tech Tip #33: Guide to enzyme substrates for ELISA
- Tech Tip #43: Protein stability and storage

Related Thermo Scientific Products

35000	**Peroxidase Suppressor,** 100mL
28320	**Tween-20 Surfact-Amps Detergent Solution,** 6 × 10mL
34065	**Metal Enhanced DAB Substrate Kit**
32020	**ABC Standard Peroxidase Staining Kit,** contains avidin and biotinylated HRP
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

Tween is a trademark of Croda International Plc.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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