INSTRUCTIONS

DAB Substrate

34001 34002

0142.3

Number	Description
34001	**DAB** (3,3´-diaminobenzidine tetrahydrochloride), 10g **Storage:** Upon receipt store product at -20°C protected from moisture. Product is shipped at ambient temperature.
34002	**DAB Substrate Kit** **Kit Contents:** **DAB Solution (10X),** 25mL **Stable Peroxide Substrate Buffer,** 250mL **Storage:** Upon receipt store kit at 4°C. Kit is shipped at ambient temperature.

Introduction

The Thermo Scientific DAB (3,3´-diaminodbenzidine) is a sensitive colorimetric substrate used with horseradish peroxidase (HRP) in immunohistochemistry and immunoblotting applications. HRP catalyzes hydrogen peroxide oxidation of substrates such as DAB. Electrons are transferred by HRP from the DAB to the peroxide to yield an insoluble brown product.

Note: Please refer to the material safety data sheet for DAB handling information.

Example Procedure for Immunohistochemical Staining

This protocol is a general guideline for using DAB 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.
- Do not use sodium azide as a preservative for buffers. Sodium azide inhibits HRP activity and could interfere with detection.
- Discard diluted and used solutions along with excess buffer after use. Do not reuse solutions.
- To prevent evaporation, use a humidity chamber set at 20-25°C for incubations. Additionally, completely cover the tissue section with solution during incubations to prevent drying. For overnight incubations, completely submerge slides and incubate at 2-8°C.
- Avoid touching slides and do not allow dust or other debris to contaminate samples, tissues or other material.
- Adjust the standard protocol when antigen concentrations are too high or low. High antigen concentrations will require less incubation time to obtain optimal staining. To shorten 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

- Cryostat sections fixed in acetone for 10 minutes and air-dried.

  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.

- 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 with some systems.

- Blocking Buffer: Normal serum with 0.05% Tween-20, 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.

- DAB Substrate: DAB is supplied either as a powder or a 10X solution.

  - For the powder, allow the bottle to warm to room temperature before opening. Dissolve DAB in 50mM Tris, pH 7.2 at 1mg/mL. Immediately before use, add an equal volume of 0.02% hydrogen peroxide.

  - For the 10X DAB solution, immediately before use, combine 2.5mL of DAB with 22.5mL of the Stable Peroxide Substrate Buffer and mix thoroughly.

C. Method

1. Quench endogenous peroxidase activity by incubating tissue for 30 minutes in Thermo Scientific Peroxidase Suppressor (Product No. 35000) or 0.3% hydrogen peroxide in methanol.

   Note: Omit this step if endogenous activity is not a problem or if the antigen will not survive exposure to H_2O_2.

2. Wash tissue with PBS-T.
3. Add Blocking Buffer and incubate for 30-60 minutes at room temperature.

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

4. Incubate tissue with the primary antibody for 30-90 minutes.
5. Wash tissue three times for 10 minutes each with PBS-T.
6. Incubate slide with HRP-labeled secondary antibody for 30 minutes.
7. Wash slide three times for 10 minutes each with PBS-T.
8. Add the hydrogen peroxide to the DAB solution (see Materials Required Section). Add the substrate solution to the sections and incubate for 2-7 minutes.
9. Wash slide two times for 3 minutes each with PBS-T. Counterstain if desired.
10. Mount slides and add a coverslip to the section after it has dried.

Note: For optimal photographic images, turn the slide upside down and place on absorbent paper. Use gentle pressure to expel excess mounting medium, wipe the edge of the slide, and seal the coverslip with clear nail polish. This procedure will produce tissue uniformity and result in optimal photographs.

Example Procedure for Western Blot Detection

This protocol is a general guideline for using DAB for Western blot detection. Optimal conditions for each specific system must be determined empirically.

A. Materials Required

- Membrane containing transferred proteins
- Phosphate Buffered Saline with Tween-20 (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 Surfact-Amps Detergent Solution (Product No. 28320), which is a specially purified Tween-20 that is free of peroxides and carbonyls that may interfere with some systems.
- Blocking Buffer: 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. Empirically determine the optimal dilution for each specific system.
- HRP-conjugated secondary antibody diluted with Blocking Buffer. Empirically determine the optimal dilution for each system being tested.
- DAB Substrate: DAB is supplied either as a powder or a 10X solution.
  - For the powder, allow the bottle to warm to room temperature before opening. Dissolve DAB in 50mM Tris, pH 7.2 at 1mg/mL. Immediately before use, add an equal volume of 0.02% hydrogen peroxide.
  - For the 10X DAB solution, immediately before use, combine 2.5mL of DAB with 22.5mL of the Stable Peroxide Substrate Buffer and mix thoroughly.

B. Procedure

1. Add Blocking Buffer to the membrane and incubate for 10-30 minutes at room temperature with shaking.
2. Decant the Blocking Buffer. 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 hydrogen peroxide to the DAB solution (see Materials Required Section). Add the DAB Substrate Solution to the membrane and incubate until the desired development is achieved. Typical incubations are from 5 to 15 minutes.

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
88013	**Nitrocellulose Membrane, 0.2µm, 7.9 × 10.5cm,** 15 sheets/pkg
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

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