INSTRUCTIONS

Thermo SCIENTIFIC

CN/DAB Substrate Kit

34000

0372.3

Number	Description
34000	**CN/DAB Substrate Kit**
	Kit Contents:
	4-Chloro-1-naphthol /3,3´-Diaminobenzidine, tetrahydrochloride (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 CN/DAB Substrate Kit is for chromogenic detection of horseradish peroxidase in immunohistochemistry and immunoblotting applications. The formed product is a black precipitate that photographs well and is useful in double-staining applications. The colored product is soluble in xylenes but not soluble in aqueous buffer such as Tris or phosphate. This combination of DAB and CN has a synergistic effect that results in greater sensitivity than either reagent alone.

Example Procedure for Immunohistochemical Staining

This protocol is a general guideline for using CN/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. Avoid touching slides and do not allow dust or other debris to contaminate samples, tissues or other material.
- Discard diluted and used solutions along with excess buffer after use.
- 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 Detergent

  Note: Use only high-quality Tween-20 Detergent such as Thermo Scientific Tween-20 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.
- Blocking buffer (e.g., Thermo Scientific StartingBlock PBS Blocking Buffer, Product No. 37538) with 0.05% Tween-20. StartingBlock™ T20 (PBS) Blocking Buffer (Product No. 37539) 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.

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C. Method

1. 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.

2. 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.

3. Wash tissue with PBS. Add blocking buffer and incubate for 30-60 minutes at room temperature.
4. Apply the primary antibody to the tissue and incubate for 30-90 minutes.
5. Rinse tissue three times for 10 minutes with PBS. Apply the HRP-labeled secondary antibody to the tissue and incubate for 30 minutes.
6. Rinse slide three times for 10 minutes with PBS.
7. Combine 2.5mL of the 10X CN/DAB Concentrate with 22.5mL of the Stable Peroxide Substrate Buffer. Mix thoroughly, filter and use immediately.
8. Apply the substrate solution to the slide. Incubate slides for 2-7 minutes or until significant color develops. To stop the reaction, wash section for 5 minutes with water.

Example Procedure for Western Blot Detection

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

A. Materials Required

- 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 (Product No. 28320)

  Note: Use only high-quality Tween-20 such as Tween-20 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.

- Blocking buffer (e.g., SuperBlock® Blocking Buffer – Blotting; Product No. 37517) with 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

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. Add the HRP-conjugated secondary antibody and incubate membrane for 1 hour at room temperature with shaking.
4. Wash membrane with PBS-T.
5. Combine 2.5mL of the 10X CN/DAB Concentrate with 22.5mL of the Stable Peroxide Substrate Buffer and mix thoroughly. Filter the solution and immediately apply it to the membrane. Observe color development.
6. Stop the reaction by rinsing membrane with water. Photograph the membrane immediately, as the color development from the CN/DAB substrate is not permanent.

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
- Tech Tip #61: De-paraffinize tissue samples for staining or immunohistochemical procedures

Related Thermo Scientific Products

35000	**Peroxidase Suppressor,** 100mL
28320	**Tween-20 Surfact-Amps Detergent Solution,** 6 × 10mL
34065	**Metal Enhanced DAB Substrate Kit,** contains 10X Metal Enhanced DAB concentrate and 1X Stable Peroxide Buffer
32020	**ABC Standard Peroxidase Staining Kit,** contains avidin and biotinylated HRP
88013	**Nitrocellulose Membrane, 0.2µm, 7.9 × 10.5cm,** 15 sheets/pkg
26681	**Pierce Blue Prestained Protein Molecular Weight Marker**
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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