INSTRUCTIONS

Chloronaphthol

34010 34011 34012

0146.2

Number	Description
34010	**4-Chloro-1-naphthol,** 25g **Storage:** Upon receipt store product at 4°C. Product is shipped at ambient temperature.
34011	**4-Chloro-1-naphthol,** 50 tablets (30mg/tablet) **Storage:** Upon receipt store tablets frozen at -20°C. Tablets are shipped at ambient temperature.
34012	**1-Step™ Chloronaphthol**, 250mL **Storage:** Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific Chloronaphthol (4-CN) is a peroxidase substrate used for chromogenic detection of HRP in immunoblotting and immunohistochemical applications. This product is less sensitive than other precipitating substrates, but the distinct blue to blue-purple precipitate photographs well and can be useful in double-staining applications. The colored product is soluble in alcohol and xylenes, but is not soluble in aqueous buffers. The Thermo Scientific 1-Step Chloronaphthol is supplied ready to use. When supplied as a powder or tablets, the substrate must be dissolved in ethanol or methanol before adding to an aqueous buffer.

Example Procedure for Immunohistochemical Staining

This protocol is a general guideline for using 4-CN 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 as it inhibits HRP activity.
- Discard diluted and used solutions along with excess buffer after use.
- 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.
- Avoid touching slides and do not allow dust or other debris to contaminate samples, tissues or other material.
- 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 be used to 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 such as Thermo Scientific 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) containing 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.
- 4-CN Substrate: The 1-Step Chloronaphthol is supplied ready to use. When using the powder or tablets, avoid moisture condensation inside the container by equilibrating the container to room temperature before opening. To make a stock solution, dissolve 4-CN in ethanol or methanol at 3mg/mL or one tablet in 10mL of ethanol or methanol. Store stock solution at -20°C for up to one year. To prepare the working solution, add 1mL of stock solution to 10mL of PBS. Immediately before use, add 0.1mL of 3% hydrogen peroxide.

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.

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

3. Wash tissue with PBS.
4. 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 with solution during incubations to prevent drying.

5. Incubate tissue with the primary antibody for 30-90 minutes.
6. Rinse tissue three times for 10 minutes with PBS.
7. Incubate slide with HRP-labeled secondary antibody for 30 minutes.
8. Rinse slide three times for 10 minutes each with PBS.
9. Add hydrogen peroxide to the substrate working solution and apply 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.
10. Counterstain if desired. Photograph immediately, as the color development from 4-CN substrate is not permanent.

Example Procedure for Western Blot Detection

This protocol is a general guideline for using 4-CN 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

  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.

- Thermo Scientific 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 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.

- 4-CN Substrate: The 1-Step Chloronaphthol is supplied ready to use. When using the powder or tablets, avoid moisture condensation inside the container by equilibrating container to room temperature before opening. To make a stock solution, dissolve 4-CN in ethanol or methanol at 3mg/mL or one tablet in 10mL of ethanol or methanol. Store stock solution at -20°C for up to one year. To prepare the working solution, add 1mL of stock solution to 10mL of PBS. Immediately before use, add 0.1mL of 3% hydrogen peroxide.

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.
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 substrate working solution and apply solution to the membrane. Incubate membrane for approximately 30 minutes at room temperature.
7. Observe color development. Stop the reaction by rinsing membrane with water. Photograph the membrane immediately, as the color development from 4-CN substrate is not permanent.

Related Thermo Scientific Products

35000	**Peroxidase Suppressor,** 100mL
28320	**Tween-20 Surfact-Amps 20 Detergent Solution,** 6 × 10mL
34065	**Metal Enhanced DAB Substrate Kit**
32020	**ABC Standard Peroxidase Staining Kit**
88018	**Nitrocellulose Membrane, 0.45µm, 33cm × 3m,** 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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