INSTRUCTIONS

Chemiluminescent Nucleic Acid Detection Module

89880

1513.4

Number	Description
89880	**Chemiluminescent Nucleic Acid Detection Module,** contains sufficient detection reagents for approximately 1000cm^2 of membrane

Kit Contents:

Stabilized Streptavidin-Horseradish Peroxidase Conjugate, 1.5mL

Chemiluminescent Substrate, stable for six months at room temperature or one year at 4°C

Luminol/Enhancer Solution, 80mL

Stable Peroxide Solution, 80mL

Blocking Buffer, 500mL

4X Wash Buffer, 500mL

Substrate Equilibration Buffer, 500mL, store at room temperature or 4°C

Storage: Upon receipt store product at 4°C. Product is shipped with an ice pack.

Introduction

The Thermo Scientific Chemiluminescent Nucleic Acid Detection Module is a complete system for the detection of biotin-labeled nucleic acids for various blotting applications including Northern/Southern blots, ribonuclease protection assays (RPAs), and electrophoretic mobility shift assays (EMSAs) or gel-shift assays. This module provides the essential detection components for the Thermo Scientific North2South Kit and Pierce Chemiluminescent EMSA Kit for the above-mentioned blotting applications. The system uses an enhanced luminol substrate for horseradish peroxidase (HRP) with optimized blocking and wash steps that together produce sensitivity equivalent to radioactive (^{32}P) systems and provide a clean, consistent background required for publication-quality data.

Additional Materials Required

- X-ray film (see Related Thermo Scientific Products) or CCD imaging instrument
- Plastic forceps
- Plastic weigh boats or other dishes

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Procedure for Detection of Immobilized Nucleic Acids

The reagent volumes indicated are for a 10 × 10cm membrane. If larger or smaller membranes are used, adjust volumes accordingly. Perform all blocking and detection incubations in clean trays or in plastic weigh boats on an orbital shaker.

1. Slowly warm the Blocking Buffer and the 4X Wash Buffer to 37-50°C in a water bath until all particulates are dissolved. These buffers may be used between room temperature and 50°C provided all particulate remains in solution. The Substrate Equilibration Buffer may be used between 4°C and room temperature.
2. To block membrane, add 16mL Blocking Buffer and incubate for 15 minutes with gentle shaking.
3. Prepare conjugate/blocking buffer solution by adding 50µL of the Stabilized Streptavidin-Horseradish Peroxidase Conjugate to 16mL Blocking Buffer (1:300 dilution).

   Note: This conjugate/blocking buffer solution has been optimized for the Chemiluminescent Nucleic Acid Detection Module and should not be modified.
4. Decant blocking buffer from the membrane and add 16mL of the conjugate/blocking solution. Incubate membrane in the conjugate/blocking buffer solution for 15 minutes with gentle shaking.
5. Prepare 1X wash solution by adding 40mL of 4X Wash Buffer to 120mL ultrapure water.
6. Transfer membrane to a new container and rinse briefly with 20mL of 1X wash solution.
7. Wash membrane four times for 5 minutes each in 20mL of 1X wash solution with gentle shaking.
8. Transfer membrane to a new container and add 30mL of Substrate Equilibration Buffer. Incubate membrane for 5 minutes with gentle shaking.
9. Prepare Chemiluminescent Substrate Working Solution by adding 6mL Luminol/Enhancer Solution to 6mL Stable Peroxide Solution.

   Note: Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Short-term exposure to typical laboratory lighting will not harm the Working Solution.
10. Remove membrane from the Substrate Equilibration Buffer and carefully blot an edge of the membrane on a paper towel to remove excess buffer. Place membrane in a clean container or onto a clean sheet of plastic wrap placed on a flat surface.
11. Pour the Substrate Working Solution onto the membrane so that it completely covers the surface. Alternatively, the membrane may be placed nucleic acid side down onto a puddle of the Working Solution. Incubate membrane in the substrate solution for 5 minutes without shaking.
12. Remove membrane from the Working Solution and blot an edge of the membrane on a paper towel for 2-5 seconds to remove excess buffer. Do not allow the membrane to become dry.
13. Wrap the moist membrane in plastic wrap, avoiding bubbles and wrinkles.
14. Expose membrane to an appropriately equipped CCD camera, or place the membrane in a film cassette and expose to X-ray film for 2-5 minutes. Develop the film according to manufacturer's instructions. Shorter or longer exposures may be used to obtain the desired signal.

Additional Information Available from the Website

- Product information and instructions for the Thermo Scientific LightShift Chemiluminescent EMSA Kit (Product No. 20148) and North2South® Hybridization and Detection Kit (Product No. 17097)
- Tech Tip #28: Stripping Northern and Southern blots
- Tech Tip #45: Anneal complementary pairs of oligonucleotides
- Frequently Asked Questions (FAQ) for the LightShift® Chemiluminescent EMSA Kit

Troubleshooting

Problem	Possible Cause	Solution
High background	Particulate in blocking buffer or wash buffer	Gently warm until no particulate remains
Speckling/spots	Precipitate in HRP conjugate	Filter the conjugate through a 0.2µm filter or centrifuge 1 minute at maximum speed
	Air bubbles	Eliminate bubbles between gel and membrane before transfer
No bands detected/low signal	Poor labeling efficiency of target or probe	Check labeling procedure for the specific assay
	Not enough biotin-labeled target or probe used	Increase target or probe concentration and/or increase film exposure time
	Target nucleic acid degraded	Check integrity of target nucleic acid
	Poor transfer to membrane	Check transfer protocol
	Wrong type of membrane used	Use Thermo Scientific Biodyne B positively charged nylon membrane (see Related Thermo Scientific Products)
	Blot dried during detection steps	Cover membrane completely during incubations
	Did not crosslink/poor crosslinking of nucleic acid to positive nylon membrane	Check efficiency of crosslinking
	Wash Buffer not diluted	Dilute 4X Wash Buffer to 1X
	Inactive HRP conjugate	Test HRP by spotting 1µL of the HRP conjugate onto a small piece of membrane, wash and detect with the substrate
	Insufficient film exposure	Increase exposure time

Related Thermo Scientific Products

20158 **LightShift Chemiluminescent RNA EMSA Kit**

20160 **RNA 3´ End Biotinylation Kit**

34090 **CL-XPosure™ Film (5" × 7" sheets)**, 100 sheets per package

21065 **Pierce Background Eliminator Kit,** for eliminating background from X-ray film

77016 **Biodyne® B Nylon Membrane**, 8 × 12cm, 0.4µm pore size, 25 sheets per package

20148 **LightShift Chemiluminescent EMSA Kit**

20148X **LightShift EMSA Optimization and Control Kit**

17097 **North2South Hybridization and Detection Kit**

89818 **Biotin 3´ End DNA Labeling Kit**, components for 20 labeling reactions

78833 **NE-PER® Nuclear and Cytoplasmic Extraction Reagents**

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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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