INSTRUCTIONS

North2South® Chemiluminescent Hybridization and Detection Kit

17097

1758.1

Number	Description
17097	**North2South® Chemiluminescent Hybridization and Detection Kit,** contains sufficient reagents for 1,000 cm^2 of membrane
	Kit Contents:
	Stabilized Streptavidin-Horseradish Peroxidase Conjugate, 1.5 ml
	Chemiluminescent Substrate, stable for six months at room temperature or one year at 4°C
	Luminol/Enhancer Solution, 80 ml
	Stable Peroxide Solution, 80 ml
	Blocking Buffer, 500 ml
	4X Wash Buffer, 500 ml
	Substrate Equilibration Buffer, 500 ml, store at room temperature or 4°C
	North2South® Hybridization Buffer, 125 ml
	North2South® Hybridization Stringency Wash Buffer (2X), 375 ml
	Storage: Upon receipt store kit at 4°C. Kit is shipped at ambient temperature.

Introduction

The North2South® Chemiluminescent Hybridization and Detection Kit is a complete system for chemiluminescent detection of nucleic acids in Northern and Southern blot applications. This system combines an enhanced luminol substrate for horseradish peroxidase (HRP) with optimized hybridization and blocking conditions that ensure consistent results with sensitivity equal to or exceeding ^{32}P. This robust system greatly reduces processing and film exposure time. Post-hybridization processing has been reduced from the standard 2.5 hours to 1 hour. Film exposure times range from 0.5 to 10 minutes with the substrate emitting light with relatively constant intensity for six hours, thus allowing for multiple exposures.

Additional Materials Required

- Biotinylated nucleic acid probe (can be produced using Product No. 17075)
- Positively charged nylon transfer membrane (Product No. 77016)
- X-ray film (Product No. 34090 or 34091) or a CCD imaging instrument

Procedure for Nucleic Acid Hybridization and Detection

A. Pre-Hybridization and Hybridization

Notes:

- Equilibrate kit buffers to room temperature before use. If there is a precipitate in any of the kit buffers, heat buffer in a 37°C water bath until precipitate disappears.
- Heat incubator to appropriate temperature (e.g., 55°C for DNA hybrids or 65°C for RNA:RNA hybrids).
- Quantify probe using a spectrophotometer.[1, 2]

1. Equilibrate the North2South® Hybridization Buffer to room temperature (RT).

   Note: This buffer has been specifically optimized for use with the North2South® Kit

2. Place blot in a container such as a 50 ml centrifuge tube and add sufficient Hybridization Buffer to completely cover the blot. Use at least 0.1 ml per cm^2 of membrane.
3. Seal the container and pre-hybridize the membrane with shaking or rotating for at least 30 minutes. For DNA hybrids incubate at 55°C; for RNA:RNA hybrids incubate at 65°C.
4. While pre-hybridizing, denature the biotinylated DNA probe. Heat DNA probe at 100°C for 10 minutes and place on ice for 5 minutes.
5. After pre-hybridization, add the denatured biotinylated probe. For biotinylated RNA probes, add 3-5 ng of probe per milliliter of hybridization buffer; for biotinylated DNA probes, add ~30 ng of probe per milliliter of hybridization buffer.
6. Incubate overnight with shaking or rotating at 55°C for DNA hybrids or 65°C for RNA:RNA hybrids.

B. Stringency Washes

1. On the next day, equilibrate the North2South® Hybridization Stringency Wash Buffer (2X) to RT. Once the Wash Buffer is fully in solution, add an equal volume of sterile ultrapure water. The resulting 1X buffer contains 2X SSC/0.1% SDS.

   Note: If required use other wash conditions to increase or decrease stringency. In general, stringency increases with increasing temperature and decreasing ionic strength.

2. Wash blot three times for 15-20 minutes per wash with agitation. Use 0.2 ml of 1X Stringency Wash Buffer per cm^2 of membrane and perform washes at 55°C for DNA hybrids or 65°C for RNA:RNA hybrids.

C. Probe Detection

Note: Use clean forceps to handle only the corners of the membrane. For optimal results, rinse forceps with ethanol and allow them to dry between steps.

1. Decant the Stringency Wash Buffer and add sufficient Blocking Buffer to generously cover the membrane. Use at least 0.25 ml/cm^2 of membrane. Incubate with shaking or rotating for 15 minutes at RT.
2. Determine the amount of Streptavidin-HRP to add to obtain a 1:300 final dilution in the tube with the blot but do not add it yet. Decant a portion of the Blocking Buffer solution from the tube containing the membrane into a separate tube.
3. Add the Streptavidin-HRP conjugate from step 2 to the separated buffer. Add the buffer/Streptavidin-HRP mix to the tube containing the membrane and incubate for 15 minutes at RT with agitation.

   Note: Performing steps 2 and 3 as described prevents the undiluted conjugate from coming in direct contact with the membrane, which would produce undesirable results.

4. Dilute Wash Buffer (4X) to 1X with sterile ultrapure water. Wash the membrane four times for 5 minutes each with 1X Wash Buffer at RT with agitation. Place membrane into a clean wash container for the next step.
5. Add Substrate Equilibration Buffer to container with the blot. Use 0.25 ml/cm^2 of membrane. Incubate blot for 5 minutes at RT with agitation.

D. Substrate Development

1. Prepare the Substrate Working Solution by mixing equal volumes of the Luminol/Enhancer Solution and Stable Peroxide Solution. Prepare enough solution to completely cover the membrane (i.e., ~0.1 ml/cm^2).

   Note: The Working Solution is stable for 6 hours at room temperature. 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.

2. Place the moist membrane on a tray or a piece of plastic wrap and cover with the Substrate Working Solution. Incubate for 5 minutes at RT. Make sure the membrane is fully covered with substrate.
3. Drain substrate from the membrane surface and transfer the moist membrane to a sheet protector or wrap in clear plastic wrap. Remove any trapped air bubbles or wrinkles within the plastic wrap and blot any substrate that may have leaked at the edges.

4. Expose blot to film for 1 minute. Adjust the exposure time as needed to obtain the desired signal.
5. Develop the film according to the manufacturer's instructions.

   Note: For images that have been overexposed, or that show high background and/or speckling, use the Erase-It® Background Eliminator Kit (Product No. 21065).

   Note: For best results, use a new membrane for each hybridization procedure. If stripping and reuse of the membrane is required, test the stripping protocol by incubating the stripped membrane in Substrate Working Solution, placing it in a sheet protector and exposing it to film for at least 30 minutes. If no bands are visible upon developing the film, rinse the membrane with Wash Buffer and re-probe.

Troubleshooting

Problem	Possible Cause	Solution
High background	Probe is too concentrated	Reduce probe concentration during hybridization
	Too much HRP conjugate used	Dilute stabilized conjugate stock ≥ 1:300 in Blocking Buffer
	Undiluted probe contacted the membrane	Thoroughly mix the probe in Hybridization Buffer before allowing it to contact the membrane
	Wrong blocking or hybridization buffer was used	Use the buffers supplied with the kit
	Wrong type of membrane was used	Use Biodyne® B Nylon Membrane (Product No. 77016)
Speckling background	Precipitate in the HRP conjugate	Filter conjugate through a 0.2 µm filter before use, or centrifuge 1 minute at maximum speed, or use Erase-It® Background Eliminator (Product No. 21065)
Clear (ghost) bands	Too much HRP	Dilute the HRP conjugate an additional 25- to 50-fold
Nonspecific bands	Degradation of target nucleic acid	Prevent nuclease contamination by using nuclease-free reagents or nuclease inhibitors
	Too much target nucleic acid present	Decrease the target concentration
	Too much probe was used	Reduce probe concentration during hybridization
	Stringency washes not sufficient	Increase stringency by decreasing the SSC concentration in the wash solution to 1X or 0.1X and/or by increasing temperature
Weak or no signal	Insufficient film exposure	Expose to film for 30 minutes or longer
	HRP conjugate concentration incorrect	Use a 1:300 dilution of a stabilized conjugate stock
	Low amount of target nucleic acid present	Increase probe concentration during hybridization and/or increase film exposure time
	DNA probe not completely denatured	Heat probe to 100°C (boil) for 10 minutes and chill on ice for 5 minutes
	Poor membrane transfer	Check transfer (blotting) protocol
	Inactive HRP conjugate	Test HRP by spotting 1 µl of the HRP conjugate onto a small piece of membrane, wash and detect with the substrate
	Probe is degraded or not labeled	Test probe by spotting it onto membrane and detecting with Streptavidin-HRP as outlined in protocol
	Probe does not hybridize to target	Use Hybridization and other buffers supplied with the kit
		Reduce the hybridization temperature

Related Thermo Scientific Products

89880	**Chemiluminescent Nucleic Acid Detection Module**
37549	**North2South® Hybridization Buffer,** 125 ml
37555	**North2South® Hybridization Stringency Wash Buffer (2X),** 375 ml
17075	**North2South® Biotin Random Prime DNA Labeling Kit**
77016	**Biodyne® Membrane,** 8 × 12 cm, 25/package
34090	**CL-XPosure™ Film,** 5 × 7 in, 100 sheets/package
21065	**Erase-It® Background Eliminator Kit,** for reducing background from exposed X-ray film
17295	**North2South® Chemiluminescent Substrate for HRP**

Additional Information

Please visit the web site for additional information on the product including the following items:

- Tech Tip protocol: Strip Northern and Southern blots
- Tech Tip resource: Northern/Southern blotting technical guide

Cited References

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press.
2. Ausubel, F.M., *et al*. (1995). *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc.

Product References

Adilakshmi, T. and Laine, R.O. (2002). Ribosomal protein S25 mRNA partners with MTF-1 and La to provide a p53-mediated mechanism for survival or death. *J. Biol. Chem*. **277(6):**4147-51.

Bach, S., *et al*. (2002). Gene expression profiling in AGS cells stimulated with *Heliobacter pylori* isogenic strains (*cagA* positive or *cagA* negative). *Infection and Immunity* **70(2):**988-92.

Borst, E-M., *et al.* (1999). Cloning of the human cytomegalovirus (HCMV) genome as an infectious bacterial artificial chromosome in *Esherichia coli*: a new approach for construction of HCMV mutants. *J. Virology*. **73(10):**8320-9.

Haertel-Wiesmann, M., *et al.* (2000). Regulation of cyclooxygenase-2 and periostin by Wnt-3 in mouse mammary epithelial cells. *J. Biol. Chem.* **275(41):**32046-51.