

Nylon

Catalog no. LC2003

Instructions for Use

Description

Nylon⁺ is a high quality, 0.45 µm membrane particularly suitable for fast, high-resolution electrotransfer of nucleic acids. The major features of Nylon membrane are:

- High sensitivity for superior detection of nucleic acids
 - High binding capacity for nucleic acids
 - Intrinsic hydrophobicity for rapid wetting
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Specifications

The specifications of Nylon membrane are listed below.

Quantity:	20 membrane / filter paper sandwiches (20 transfers)
Pore Size:	0.45 µm
Dimensions:	8.3 cm x 7.3 cm
Charge:	Positive, formed by strongly cationic quaternary ammonium groups
Binding Capacity:	100 µg / cm ² of nucleic acid 200 µg / cm ² of protein
Re-probe Characteristics:	Yes

Applications

Nylon membrane is suitable for the following applications:

- Southern, Northern, and Western Transfers
 - Solid Phase Immobilization
 - Dry Chemistry Test Strips
 - Enzyme Immobilization
 - Gene Probe Assays
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Materials Needed

You will need the following items for blotting and hybridization. Ordering information is provided on the next page.

- TBE buffer
 - PBS (phosphate buffered saline)
 - 20X SSC
 - 0.5% Casein in PBS (Add 0.5 g casein to 100 ml PBS and heat the solution to 60-80°C with stirring. Avoid foaming. Once the casein is dissolved, remove from heat and filter the solution through a 0.45 µm filter.)
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This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen TECH-LINESM 800 955 6288

Instructions for Use, Continued

Procedure

All washes are done in a shallow dish with constant mixing.

1. After running and staining (optional) the gel, denature the gel for 10 minutes in 50 mM NaOH. Rinse the gel quickly in deionized water, followed by a 5 minute wash in 1X TBE. **Note:** Stain for 10 minutes with 0.2 µg/ml ethidium bromide and wash for 10 minutes in deionized water.
 2. Wet the Nylon membrane and filter paper in 1X TBE. Perform transfer using the XCell II™ Blot Module for 1.5 hours at 20 volts. If you are using any other blotting apparatus, select the necessary voltage to generate a field strength of 7 volts/cm. Use TBE Buffer at 1/2X concentration for better transfer results.
 3. After transfer is complete, immerse the membrane briefly in 0.1 N NaOH. Rinse the membrane two times with deionized water to remove the NaOH.
 4. Air dry the membrane and fix the DNA by UV irradiation (0.2 joules/cm²) or dry at 80°C for 30 minutes.
 5. Block the membrane for 30 minutes at 37°C with 0.1% SDS in PBS. Decant the SDS solution and block for 1 hour at 37°C with 0.5% casein in PBS.
 6. Probe the membrane for 2 hours or longer in 6X SSC at 40°C for typical synthetic probes.
 7. Wash the membrane 3 times for 10 minutes each with PBS (or until no background counts are detected).
 8. Air dry and perform autoradiography. **Note:** Do not allow the membrane to dry if stripping and reprobing is required (see below).
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Reprobing

To reprobe the membrane, you need to strip the probe from the membrane.

To strip the probe from the membrane, wash the membrane with two changes of hot (95-100°C) 0.1% SDS in deionized water. Shake the membrane briefly in the first change of hot 0.1% SDS solution. Decant the solution and add the second change of hot 0.1% SDS solution immediately and shake the membrane briefly. Allow the membrane to cool and proceed to blocking with the casein solution and probing as described above.

Related Products

Additional products that can be used with the Nylon membranes are available separately from Invitrogen. Ordering information is provided below. For more information, visit our Web site at www.invitrogen.com or contact Technical Service.

Product	Quantity	Catalog no.
TBE Running Buffer (5X)	1 L	LC6675
UltraPure™ SSC (20X)	1 L	15557-044
Phosphate-Buffered Saline, pH 7.2 (10X)	500 ml	70013-032
XCell II™ Blot Module	1 unit	EI9051

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