SensiBlot™ Plus Nylon Membrane

#M1001 10 sheets (20x20cm)

#M1002 1 roll (0.3x3m)

Store at room temperature

Description

The SensiBlot™ Plus Nylon Membrane is the hybridization membrane of choice for use with Fermentas radioactive and non-radioactive labeling and detection kits. These membranes ensure high sensitivity and low background in Southern blot and other blotting experiments.

The SensiBlot™ Plus Nylon Membrane is a nylon membrane with quaternary ammonium surface chemistry. Positive surface charge is maintained over a wide range of pH values. Standard pore size for SensiBlot™ Plus membrane is 0.45 µm.

The SensiBlot™ Plus Nylon Membrane guarantees high sensitivity and low background for enhanced detection and resolution in both radioactive and non-radioactive detection systems. It does not crack, shrink, or tear when subjected to multiple cycles of hybridization, stripping and re-probing. The SensiBlot™ Plus Nylon Membrane is intrinsically hydrophilic and moistens easily.

Applications

- Southern blots.
- Northern blots.
- Dot/slot blots.
- Colony and plaque hybridizations.

INSTRUCTIONS FOR USE

Guidelines for DNA Detection

Purchasers of commercially available DNA detection kits should follow manufacturer's recommendations. The following procedures are offered as guidelines for obtaining best results or developing a new detection system.

Denaturation

For most hybridization applications, single stranded (ss) nucleic acids are used. The easiest way to generate ss-DNA is to boil the solution of double-stranded DNA for 5-10 minutes, and then to chill it on ice immediately.

Immobilization

DNA can be immobilized on the membrane *using* dot/slot blotting, array printer, capillary transfer, electrotransfer or other methods. Suitable dilution buffers, like 2X SSC, 1X SSPE, should be used.
Optimum amount of DNA varies. Usually, the maximal signal is obtained at the 1 µg/µL concentration of DNA, while detection limits are typically in the range of 10-30 fg/µl. Using Southern transfers, single copy alleles are usually detected when amount of the total DNA digest is between 0.1-3µg per lane.

Fixation

Membranes should be air-dried following the immobilization step. The highest signal and sensitivity are obtained if dry membrane is incubated at 80°C for 30 minutes, and then it is UV cross-linked at 100-150mJ. Free standing UV light sources can be used for this purpose having the optimal exposure time determined empirically. Overexposure to the UV light renders the immobilized nucleic acid less recognizable.

Prehybridization *(blocking step)*

A casein based blocking solutions are strongly recommended for both the pre-hybridization and the hybridization steps (*see* below). A typical blocking solution may include the following: 2X SSC, 7% SDS and 0.5% Hammersten casein. Casein powder is dissolved by heating on a stir plate (avoid boiling). Membranes should be prehybridized for 30 minutes, or longer at 60°C.

Hybridization

The labeling efficiency and optimal probe concentration should be determined by dot blot trials prior to actual experiments.

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Membranes are typically hybridized overnight.
A convenient method is to place the membrane in a centrifuge tube, completely covered it with the hybridization solution, and incubate the tube in a hybridization oven overnight at 65 °C. The use of special hybridization tubes or sealed bags is recommended. Agitation is important during this step. The hybridization solution should move freely over the membrane.
Note. Lower hybridization temperatures may result in a higher signal, but chances of detecting false positives due to mismatches. Shorter hybridization times may also be used, particularly if the probes or targets represent short nucleotide sequences.

Stringency Washes

Wash twice with 2X SSC supplemented with 0.1% SDS at room temperature for 15minutes.

Wash twice with 2X SSC supplemented with 0.1% SDS at 60°C for 15minutes.

Rinse twice with deionized or distilled water (for radiolabeled probes), or with a conjugate buffer (without blocking agent) for non-radioactive probes

Detection with Radiolabeled Probes

Place membranes in clear plastic folders. Place the folder(s) in an autoradiography cassette containing a suitable film (Kodak X-OMat™, Amersham Hyperfilm™ MP, or their equivalent). Expose for 1-72 hours. Longer exposures should be performed at -70°C. Develop the exposed film following the manufacturer's instructions.

Detection with Non-radioactively-labeled Probes

(including biotin, DIG and FITC)

All the steps described below should be carried out at room temperature with moderate agitation.

Use enough solution to completely cover the membrane and allow its free floating.

- Block membranes in a suitable blocking solution, e.g. PBS supplemented with 0.5% Hammersten grade casein. It is usually sufficient to block the membrane for 30-60 minutes.
- Incubate (usually 15-30 minutes) the blocked membrane in a solution of a respective antibody (streptavidine) coupled to a reporter enzyme. Dilute antibody-reporter conjugates in a blocking solution according to the manufacturer's recommendations
- Wash the membrane. Two 15-minute washes with PBS, or other suitable washing buffer, are generally sufficient.
- Equilibrate the membrane with a detection buffer.

- Replace the detection buffer with the substrate solution to initiate reaction catalyzed by the reporter enzyme. ***Note.*** Examples of suitable substrates are the following: BCIP/NBT, dioxetane-based chemiluminescent substrates (Lumi-Phos™ Plus or CDP Star™), and other chemifluorescent substrates, such as Attophos™.
- When using color-forming substrates, allow the reaction to proceed until no additional DNA is detected on the membrane, or until the background color becomes noticeable.
- When using chemiluminescent substrates, incubate membranes for 2 minutes. Then, drain excess solution and seal membranes in clear plastic folders. Allow the reaction to reach steady state (30 to 60 minutes). Place folder(s) into an autoradiography cassette containing a suitable film (Kodak X-O Mat™, Amersham Hyperfilm™ MP, or equivalent). Exposure time can be 2 minutes, or less, or exposure can be prolonged up to 18 hours.

Related Products

• Biotin DecaLabel™ DNA Labeling Kit	#K0651/2
• DecaLabel™ DNA Labeling Kit	#K0621/2
• HexaLabel™ DNA Labeling Kit	#K0611/2
• HexaLabel™ Plus DNA Labeling Kit	#K0613/4
• Biotin Chromogenic Detection Kit	#K0661/2
• Aminoallyl-dUTP	#R0091/1101
• Aminoallyl-UTP	#R1091
• Biotin-11-dUTP	#R0081
• Fluorescein-12-dUTP	#R0101
• NBT	#R0821/2
• BCIP-T	#R0841/2