For life science research only.
Not for use in diagnostic procedures.

Nylon Membranes, positively charged

Cat. No. 11 209 272 001 10 sheets à 20 × 30 cm
Cat. No. 11 209 299 001 20 sheets à 10 × 15 cm
Cat. No. 11 417 240 001 1 roll à 0.3 × 3 m

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Store at +15 to +25°C

1. Product overview

Product description
Nylon membranes are microporous, positively charged, pure nylon, bound to a polyester support.

They are cationic and maintain their positive charge over a wide pH range. These membranes therefore have a high binding capacity for DNA and RNA under standard Southern-, northern- and dot-blot conditions (1, 2), as well as in alkaline transfer procedures (3, 4).

Pore size
0.45 μm

Surface properties
Hydrophilic, with positive Zeta-surface potential.

Application
The physical and chemical properties of the nylon membranes make them especially useful as a matrix for the hybridization of

- Southern blots
- northern blots and
- Dot blots

with non-radioactively [*e.g.* Digoxigenin (DIG)] or radioactively (e.g. ^{22}P, ^{35}S and ^{3}H) labeled DNA or RNA probes.

Storage/stability
Stable at +15 to +25°C until the control date printed on the label. Please store protected from light.

Storage prior to first hybridization
After blotting, you may store the blot for an unlimited amount of time before using it in a hybridization assay, as long as the membrane is kept dry, sealed in protective plastic and stored in the dark at +15 to +25°C.

2. General remarks on usage and application

General
DNA and RNA can be separated by gel electrophoresis according to standard procedures (1, 2, 3). The nylon membranes may be used in all routine Southern and Northern transfer procedures (2, 4, 5).

Handling of membrane

- The membranes are mechanically robust and resistant to tearing or cracking.
- Use scissors or a sharp scalpel to cut membranes to size.

Note: Always wear gloves or use forceps when handling membranes.

Pre-wetting of membrane
Nylon membranes do not require pre-wetting before use. However, if the membrane will immediately be in contact with solutions of high ionic strength (20 × SSC Buffer*), you should pre-wet the membrane with either double-dist. water or 2 x SSC.

Place the membrane on the surface of this solution for a few seconds then submerge it to complete the wetting process. If required, you may then place the membrane in a high salt transfer buffer for 5-15 min to equilibrate.

Alkaline transfer procedure
Due to their high DNA binding capacity, these membranes perform especially well in a modified rAPid alkaline transfer procedure where the transfer from agarose gels is performed in 0.4 M NaOH directly after electrophoresis without denaturation or neutralization steps (6,7). When using the alkali transfer method, crosslink the DNA to the membrane.
Note: Alternatively, you may perform UV crosslinking after alkaline transfer if you neutralize the membrane before exposing it to UV.

Fixation of nucleic acids
For dot blots and Southern transfers, you must bind the DNA to the membrane by either baking at +120°C for 15-30 min or UV-cross linking for approx. 3 min (trans-illuminator). You may also use the Stratalinker from Stratagene according to manufacturers instructions.

We especially recommend UV crosslinking for Northern transfers.

2.1 Hybridization and immunological detection

Hybridization with radioactively labeled probes
Hybridization with radioactively labeled DNA or RNA probes can be performed according to standard procedures with these nylon membranes (8, 9).

Note: Prehybridization and hybridization solutions should contain 100 μg/ml denatured fish sperm DNA.

Hybridization with DIG-DNA probes
Due to an homogeneous charge distribution, these membranes are especially suited for hybridization with non-radioactively labeled probes, which can be detected with chemiluminescent substrates.

For high sensitivity and the low background, we recommend using optimal amounts of DIG-labeled DNA probes and performing pre-hybridization and hybridization in DIG Easy Hyb buffer. DIG Easy Hyb buffer is non-toxic and does not contain formamide. Other commonly used hybridization solutions also work well.

Hybridization with DIG-RNA probes
For northern blots with DIG-labeled RNA probes, we recommend to use DIG Easy Hyb buffer or hybridization solutions that contain formamide. Detailed protocols are available in DIG Easy Hyb buffer package insert or in the DIG Application Manual for Filter Hybridization (available on request).

The optimal concentration of the labeled RNA in the hybridization mixture depends on the amount of DNA or RNA to be detected on the filter. We recommend using not more than 100 ng of labeled RNA per ml hybridization solution.

Immunological detection
For immunological detection of DIG labeled probes, use the highly specific anti-DIG –AP antibody* and chemiluminescent or color substrates for alkaline phosphatase.

For optimal sensitivity, we recommend using the chemiluminescent substrates CDP-*Star** and CSPD* in combination with the DIG Wash and Block Buffer Set*. Details and protocols are available in the package inserts of these substrates.

2.2 Stripping and rehybridization

Note: Always prepare the stripping buffer shortly before use. Do not let the membrane dry out at any time. Stripping and reprobing can been done repeatedly for many times.

2.2.1 Stripping and rehybridization of DNA: DNA Hybridization probes labeled with alkali-labile DIG dUTP

Additional reagents required

- PCR grade water
- 0.2 N NaOH, SDS, 0.1% (w/v)
- 2 × SSC

Procedure

Step	Action
1	Rinse membrane briefly in PCR grade water.
2	Wash for 2 × 15 min in 0.2 N NaOH, SDS, 0.1% (w/v) at +37° C under constant agitation.
3	Equilibrate briefly in 2 × SSC.
4	Prehybridize and incubate with second probe.

2.2.2 Stripping DIG-labeled RNA probes (Northern blot)

Additional reagents required

- PCR grade water
- 2 × SSC

Stripping buffer 50% deionized formamide, 5% SDS, 50 mM Tris-HCl, pH 7.5.

Procedure

Step	Action
1	Rinse membrane thoroughly in PCR grade water.
2	Wash membrane at +80° C in Stripping Buffer for 2× 60 min.
3	Rinse membrane thoroughly in 2 × SSC for 5 min.

3. References

1 Maniatis, T. *et al.,*(1982) in Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, New York
2 Southern, E. M. (1975) *J- Mol. Biol.* **98**, 503--517.
3 Thomas, P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201-6206
4 Wahl, G.M, Stern, M. & Stark, G.R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3683-3687.
5 Alwine, J.C. *et al.,* (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5350.
6 Chornczynski, P. & Qasha, P.K. (1984) *Biochem. Biophys. Res. Commun.* **122,** 340-344
7 Reed, K C. & Mann, D. A. (1985) *Nucleic Acids Res.* **13**, 7207-7221
8 Nucleic acid hybridization, a practical approach (1985) (Hames, B.D. & Higgins,S.J., eds.) IRL Press Ltd., Oxford, U. K
9 Meinkoth, J, & Wahl, G. (1984) *Anal. Biochem.* **138**, 267-284.
10 DIG Application Manual for Filter Hybridization (2000) (Roche Diagnostics)

3.1 Printed materials

You can view the following manuals on our website:

DIG Product Selection Guide
DIG Application Guide for Filter Hybridization
Nonradioactive *In Situ* Hybridization Manual

3.2 Ordering Information

Kits

Product	Pack Size	Cat. No
DIG High Prime DNA Labeling and Detection Starter Kit I	12 labeling reactions and 24 blots	11 745 832 910
DIG High Prime DNA Labeling and Detection Starter Kit II	12 labeling reactions and 24 blots	11 585 614 910
DIG Northern Starter Kit	1 kit	12 039 672 910
DIG DNA Labeling Kit	40 labeling reactions	11 175 033 910
DIG RNA Labeling Kit (SP6/T7)	2 × 10 reactions	11 277 073 910
DIG Oligonucleotide 3'-End Labeling Kit, 2nd Generation	25 labeling reactions	03 353 575 910
DIG Oligonucleotide Tailing Kit, 2nd Generation	25 reactions	03 353 583 910
DIG DNA Labeling and Detection Kit	25 labeling reactions and 50 color detections (blots :10 × 10 cm)	11 093 657 910
DIG Nucleic Acid Detection Kit (Color detection)	40 blots (10 × 10 cm)	11 175 041 910
DIG Luminescent Detection Kit for Nucleic Acids	50 blots (10 × 10 cm)	11 363 514 910
DIG Wash and Block Buffer Set	1 set	11 585 762 001

Single reagents

Product	Pack Size	Cat. No.
DIG-High Prime	160 µl (40 reactions)	11 585 606 910
Biotin High Prime	100 µl (25 reactions)	11 585 649 910
DIG Easy Hyb	500 ml	11 603 558 001
DIG Easy Hyb Granules	6 × 100 ml	11 796 895 001
Nylon Membranes for Colony and Plaque Hybridization	50 filters (∅ 82 mm) 50 filters (∅ 132 mm)	11 699 075 001 11 699 083 001
Blocking reagent	50 g	11 096 176 001
Buffers in a Box, Premixed SSC Buffer, 20×	4 l	11 666 681 001
Anti-Digoxigenin-AP, Fab fragments	150 U (200 µl)	11 093 274 910
NBT/BCIP	8 ml	11 681 451 001
NBT/BCIP ready-to-use tablets	20 tablets	11 697 471 001
CSPD	1 ml	11 655 884 001
CSPD ready-to-use	2 × 50 ml	11 755 633 001
CDP-*Star*	1 ml 2 × 1 ml	11 685 627 001 11 759 051 001
CDP-*Star*, ready-to-use	2 × 50 ml	12 041 627 001
Hybridization bags	50 bags	11 666 649 001

*available from Roche Diagnostics

Changes to Previous Version Editorial changes.

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