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

Nylon Membranes, positively charged

Version: 21
Content Version: June 2021

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

Store the product at +15 to +25°C.

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1. General Information

1.1. Contents

Package	Label	Function / Description	Catalog Number	Content
1	Nylon Membranes, positively charged	▪ Microporous, positively charged, pure nylon bound to a polyester support. ▪ Surface properties: hydrophilic with positive zeta-surface potential. ▪ Pore size 0.45 µm.	11 209 272 001	10 sheets, 20 × 30 cm
			11 209 299 001	20 sheets, 10 × 15 cm
			11 417 240 001	1 roll, 0.3 × 3 m

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the product is stable through the expiry date printed on the label.

Package	Label	Storage
1	Nylon Membranes, positively charged	Store at +15 to +25°C. ⚠ ***Keep protected from light.***

Storage Conditions (Working Solution)

After blotting, you can store the membrane for an unlimited amount of time before using it in a hybridization assay.
Keep the membrane dry, sealed in protective plastic, and keep protected from light at +15 to +25°C.

1.3. Additional Equipment and Reagent required

For hybridization

- Denatured fish sperm DNA
- DIG Easy Hyb* or
- DIG Easy Hyb Granules*

For immunological detection

- Anti-Digoxigenin-AP, Fab fragments*
- CDP-*Star**
- CSPD*
- NBT/BCIP*
- DIG Wash and Block Buffer Set*

For stripping and rehybridization of DNA:DNA hybridization probes

- Water, PCR Grade*
- 0.2 M NaOH
- 0.1% SDS* (w/v)
- 2x SSC*

For stripping DIG-labeled RNA probes (northern blot)

- Water, PCR Grade*
- 2x SSC*
- Stripping buffer: 50% deionized Formamide*, 5% SDS*, 50 mM Tris-HCl*, pH 7.5

1.4. Application

The physical and chemical properties of the Nylon Membranes make them especially useful as a matrix for the hybridization of blots with nonradioactively labeled Digoxigenin (DIG) or radioactively labeled ^{22}P, ^{35}S, and ^{3}H DNA or RNA probes. It can be used for:

- Southern blots
- Northern blots
- Dot blots

DNA and RNA can be separated by gel electrophoresis according to standard procedures. The membranes can be used in all routine Southern and northern transfer procedures.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Membrane handling

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

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, such as 20x SSC Buffer*, pre-wet the membrane with either double-distilled water or 2x SSC:

1. Place the membrane on the solution surface for several seconds.
2. Submerge membrane to finalize the wetting process.
3. If required, place the membrane in a high-salt transfer buffer for 5 to 15 minutes to equilibrate.

Alkaline transfer procedure

Due to their high DNA binding capacity, the membranes perform especially well in a modified rAPid alkaline transfer procedure. Optimal transfer from agarose gels is performed in 0.4 M NaOH directly after electrophoresis without denaturation or neutralization steps. When using this method, crosslink the DNA to the membrane.

Alternatively, 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, bind the DNA to the membrane by either baking at +120°C for 15 to 30 minutes or UV crosslinking for approximately 3 minutes using a transilluminator.

Use UV crosslinking for northern transfers.

2.2. Protocols

Hybridization with radioactively labeled probes

Perform hybridization with radioactively labeled DNA or RNA probes according to standard procedures.

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

Hybridization with DIG-DNA probes

Due to a homogeneous charge distribution, these membranes are especially suited for hybridization with nonradioactively labeled probes detected with chemiluminescent substrates. For high sensitivity and low background, use optimal amounts of DIG-labeled DNA probes and perform 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, use DIG Easy Hyb buffer* or hybridization solutions that contain formamide. The optimal labeled RNA concentration in the hybridization mixture depends on the amount of DNA or RNA detected on the filter.

⚠ ***Use ≤100 ng of labeled RNA per ml hybridization solution.***

Immunological detection

For immunological detection of DIG-labeled probes, use the highly specific Anti-Digoxigenin-AP, Fab fragments antibody* and chemiluminescent or color substrates for alkaline phosphatase*. For optimal sensitivity, use the chemiluminescent substrates CDP-*Star*® and CSPD® in combination with the DIG Wash and Block Buffer Set*.

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

⚠ ***Always prepare the stripping buffer shortly before use. Never let the membrane dry out.***

ⓘ *Stripping and reprobing can been repeated multiple times.*

1. Rinse membrane briefly in Water, PCR Grade*.
2. Wash for 2 × 15 minutes in 0.2 M NaOH, 0.1% SDS*, (w/v) at +37°C under constant agitation.
3. Equilibrate briefly in 2x SSC*.
4. Prehybridize and incubate with second probe.

Stripping DIG-labeled RNA probes (northern blot)

1. Rinse membrane thoroughly in Water, PCR Grade*.
2. Wash membrane twice at +80°C in Stripping buffer for 60 minutes each time.
3. Rinse membrane thoroughly in 2x SSC for 5 minutes.

2.3. Parameters

pH Optimum

The Nylon Membranes 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 as well as in alkaline transfer procedures.

3. Supplementary Information

3.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

3.2. Changes to previous version

Layout changes.
Editorial changes.

3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris hydrochloride	500 g	10 812 846 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
NBT/BCIP Stock Solution	8 ml	11 681 451 001
NBT/BCIP Ready-to-Use Tablets	20 tablets	11 697 471 001
Formamide	500 ml	11 814 320 001
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
CSPD	1 ml	11 655 884 001
CDP-*Star*	1 ml	11 685 627 001
	2 x 1 ml	11 759 051 001
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
CDP-*Star*, ready-to-use	2 x 50 ml	12 041 677 001
DIG Easy Hyb	500 ml	11 603 558 001
DIG Easy Hyb Granules	6 bottles, Granules for 6 x 100 ml	11 796 895 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001

3.4. Trademarks

DIG EASY HYB is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

3.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

3.6. Regulatory Disclaimer

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

3.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

3.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

0621.11216813001 ©

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