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

Nylon Membranes for Colony and Plaque Hybridization

Version: 08
Content Version: June 2021

Cat. No. 11 699 075 001 50 discs
82 mm diameter

Store the product at +15 to +25°C.

1. General Information

1.1. Contents

Package	Label	Function / Description	Content
1	Nylon Membranes for Colony and Plaque Hybridization	▪ Microporous, amphoteric, ultra-pure nylon 66 cast on a polyester support. ▪ Surface properties: hydrophilic with 50% amino groups and 50% carboxyl groups. Uncharged at isoelectric point at pH 6.5. ▪ Pore size 1.2 µm.	50 discs, 82 mm Ø each

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 for Colony and Plaque Hybridization	Store at +15 to +25°C.

1.3. Additional Equipment and Reagent required

For colony and plaque lifts

See section, **Working Solution** *for additional information on preparing solutions.*

- Whatman 3MM paper
- Agarose MP*
- NaCl, NaOH, Na citrate
- Tris-HCl*
- 2x SSC*
- Proteinase K*
- Foil
- Tray
- Double-distilled water
- YT medium

For hybridization

- Roller bottles
- DIG Easy Hyb*
- 10% SDS* (w/v)
- 2x SSC*
- Hybridization oven

For nonradioactive labeling of DNA probes

- PCR DIG Probe Synthesis Kit*
- DIG-High Prime*
- Biotin-High Prime*, or
- Fluorescein-High Prime*

For stringency washes after hybridization

- 2x SSC*
- 0.1% SDS*
- 0.5x SSC*

For detection

- Hybridization bags
- Anti-Digoxigenin-AP, Fab fragments*, or
- Anti-Fluorescein-AP*, or
- Streptavidin-AP*

For chemiluminescent detection with CSPD

See section, **Working Solution** *for additional information on preparing solutions.*

- Washing buffer
- Maleic acid buffer
- Detection buffer
- Blocking Reagent*

The Washing buffer, Maleic acid buffer, Detection buffer, and Blocking reagent are available in the DIG Wash and Block Buffer Set, DNase and RNase free.*

- CSPD* or CSPD, ready-to-use*
- Lumi-Film Chemiluminescent Detection Film*

For removal of probe

- Double-distilled water
- 0.2 M NaOH
- 0.1% SDS*
- 2x SSC*

For color detection

- NBT/BCIP stock solution*
- TE buffer or double-distilled water
- Washing buffer
- Maleic acid buffer
- Detection buffer
- Blocking Reagent*

The Washing buffer, Maleic acid buffer, Detection buffer, and Blocking reagent are available in the DIG Wash and Block Buffer Set, DNase and RNase free.*

For stripping of color substrate

- Dimethylformamide (DMF)
- Double-distilled water
- 0.2 M NaOH
- 0.1% SDS*
- 2x SSC*

1.4. Application

The Nylon Membranes for Colony and Plaque Hybridization are suitable for all routine colony and plaque lift procedures:

- Physical and chemical properties of the membranes make them especially useful for plaque and colony lifts directly from agar plates.
- 1.2 µm pore size allows a rapid and quantitative transfer and binding of DNA from lysed plaques or colonies.
- Extremely high binding capacity enables highly sensitive detection of labeled probes after hybridization.
- Membrane discs are especially suited and tested for nonradioactive screening of phage or cosmid libraries with digoxigenin (DIG)-labeled probes and detection with highly sensitive chemiluminescent (CSPD*, CDP-*Star*®) or chromogenic substrates (NBT/BCIP®).
- Optimized retention of nucleic acids and the mechanical robustness allow multiple stripping and reprobing with different probes.

The transferred DNA is crosslinked to the membrane by UV light or baking at +80°C.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Membrane handling

To avoid contamination and background formation during hybridization and detection:
- Use a pair of filter-tweezers to handle the membranes.

Always wear gloves when handling membranes.

Pre-wetting of membrane

The membrane discs do not require pre-wetting before use; use directly for colony or plaque lifts.

Hybridization container

- Hybridize membrane discs in roller-bottles or sealed in hybridization bags.
- When glass or petri dishes are used, close tightly.

Membranes must not stick to each other and must be sufficiently covered with hybridization solution.

Hybridization temperature

The appropriate hybridization temperature is calculated according to G/C content and percent homology of probe to target DNA with the following equation:

Tm = 49.82 + 0.41 (% G+C) – (600/l)
l = length of hybrid in bp
T_{opt} = Tm – (+15 to +25°C)

The actual hybridization temperature T_{opt} with DIG Easy Hyb is +15 to +25°C below the calculated Tm value. T_{opt} can be regarded as a stringent hybridization temperature, allowing up to 18% mismatches between probe and target.

When the degree of homology of your probe to template is less than 80%, lower the T_{opt} accordingly (approximately 1.4°C below Tm per 1% mismatch), and also adjust the stringent washing steps accordingly, that is, increase SSC concentration and lower washing temperature.

Working Solution

Colony and Plaque Lifts		
Solution	**Composition/Preparation**	**For use in...**
Denaturation solution	0.5 M NaOH, 1.5 M NaCl	Denaturing bound material on the membrane.
Neutralization solution	1.5 M NaCl, 1.0 M Tris-HCl*, pH 7.4, (+21°C)	Neutralizing the membrane.
2x SSC	0.3 M NaCl, 0.3 M Na citrate, pH 7.0	Soaking filter and dilution of Proteinase K.
Proteinase K solution	Dilute Proteinase K* (>600 U/ml, 14 to 22 mg/ml) 1:10 in 2x SSC*.	Removing cellular debris.

Detection			
Solution	**Composition/Preparation**	**Storage and Stability**	**For use in...**
Washing buffer	0.1 M maleic acid, 0.15 M NaCl, pH 7.5 (+15 to +25°C), 0.3% (v/v) Tween 20*	Store at +15 to +25°C.	Washing of membrane.
Maleic acid buffer	0.1 M maleic acid, 0.15 M NaCl; adjust with solid NaOH to pH 7.5 (+15 to +25°C)		Dilution of Blocking solution.
Detection buffer	0.1 M Tris-HCl*, 0.1 M NaCl, pH 9.5 (+15 to +25°C)		▪ Alkaline phosphatase buffer. ▪ For dilution of color substrate solution and CDP-*Star**.
Blocking stock solution, 10x conc.	Dissolve Blocking Reagent* 10% (v/v) in Maleic acid buffer under constant stirring on a heating block, or heat in a microwave oven and autoclave. *The solution remains opaque.*	Store at +2 to +8°C initially; after first usage, store in aliquots at −15 to −25°C.	Preparation of Blocking solution.
Blocking solution, 1x conc.	Dilute the 10x Blocking solution 1:10 with Maleic acid buffer (final concentration 1%).	***Always prepare fresh.***	Blocking of nonspecific binding sites for digoxigenin-labeled probes.
Blocking solution, 5x conc.	Dilute 10x Blocking solution 1:2 in Maleic acid buffer (final concentration 5%).	***Always prepare fresh.***	Blocking of nonspecific binding sites for biotin-labeled probes.
Chemiluminescent Detection with CSPD			
Solution	**Composition/Preparation**	**Storage and Stability**	**For use in...**
CSPD, 1x conc.	Dilute CSPD* 100x conc. 1:100 in Detection buffer. *Alternatively, use CSPD, ready-to-use**.	Store at +2 to +8°C. ***Keep protected from light.*** ***Can be used one to two times when kept sterile.***	Chemiluminescent reaction.
Antibody solution	▪ Centrifuge the antibody for 5 minutes at 10,000 rpm in the original vial prior to each use, and pipette the necessary amount carefully from the surface. ▪ Dilute Anti-Digoxigenin-AP, Fab fragments* 1:10,000 (75 mU/ml) in 1x Blocking solution.	Store at +2 to +8°C for 12 hours.	Binding to the DIG-labeled probe.
Color Substrate Detection with NBT/BCIP			
Solution	**Composition/Preparation**	**Storage and Stability**	**For use in...**
Color substrate solution, 1x conc.	80 µl NBT/BCIP* stock solution in 4 ml Detection buffer.	***Always prepare fresh.***	Color reaction.
Antibody solution	▪ Centrifuge the antibody for 5 minutes at 10,000 rpm in the original vial prior to each use, and pipette the necessary amount carefully from the surface. ▪ Dilute Anti-Digoxigenin-AP, Fab fragments* 1:5,000 (150 mU/ml) in 1x Blocking solution.	Store at +2 to +8°C.	Binding to the DIG-labeled probe.
TE buffer	10 mM Tris-HCl*, 1 mM EDTA, pH 8.0 (+15 to +25°C)	–	Stopping the color reaction.

*The Washing buffer, Maleic acid buffer, Blocking solution, and Detection buffer are available DNase- and RNase-free in the DIG Wash and Block Buffer Set**.

2.2. Protocols

Colony and plaque lifts

i *See section,* **Working Solution** *for additional information on preparation of solutions.*

1. Soak 2 layers of Whatman 3MM paper for each solution: Denaturation, Neutralization, and 2x SSC.

2. Pre-cool colonies or plaques on agar plates for approximately 30 minutes at +2 to +8°C.
   i *For plaque lifts, use Agarose MP* for top agar (0.7% in YT medium).*

3. Carefully place a membrane disc onto the surface, avoiding bubbles.
   ⚠ ***Do not move the membrane once it has attached.***

4. Leave the membrane disc on the plate for approximately 1 minute.
   – Mark the orientation to the plate in order to identify positive plaques or colonies.

5. Remove membrane disc carefully with filter tweezers and briefly blot the upside of the colonies or plaques on Whatman 3MM paper.

6. Place the upside of the membrane disc onto the prepared filter paper soaked with Denaturation solution (15 minutes for colony lifts or 5 minutes for plaque lifts).
   – Air dry on Whatman 3MM paper.

7. Place membrane disc onto the prepared filters soaked with Neutralization solution for 15 minutes.
   – Air dry on Whatman 3MM paper.

8. Place membrane disc onto the prepared filters soaked with 2x SSC* for 10 minutes.
   – Subsequently, crosslink the transferred DNA with UV light for approximately 1 minute or use a commercially available crosslinking device.
   i *Alternatively, bake the dry membranes for ≥30 minutes at +80°C.*

9. To remove cellular debris from colony lifts, place membrane disc onto a foil and pipette 0.5 ml Proteinase K* solution (1:10) onto each 82 mm Ø membrane disc; distribute evenly.
   – Incubate for 1 hour at +37°C.
   ⚠ ***Ventilation in the incubator must be turned off.***

10. Pre-wet Whatman 3MM paper with double-distilled water.
    – Place all filters on solid support, such as a tray.
    – Cover with the Whatman paper and firmly press across the paper using a ruler or other firm device.
    – Remove debris by gently pulling off the Whatman paper.
    i *The cellular debris will stick to the filter paper. If debris remains on the membrane discs, repeat procedure.*

11. Proceed with the hybridization procedure.

Hybridization

The following volumes are calculated for the use of a 275 ml volume roller bottle. The hybridization temperature is given for a 100% homologous probe with 50% G/C content.

1. Place 3, 82 mm Ø membrane discs in a roller bottle and add 60 ml DIG Easy Hyb* buffer.
   – Prehybridize for 1 hour at +42°C in a hybridization oven for roller bottles.

2. Denature 25 ng/ml labeled probe by boiling for 5 minutes at +95 to +100°C and rapidly place on ice.

3. Mix 5 to 25 ng/ml denatured probe with DIG Easy Hyb buffer, prewarmed to hybridization temperature.

4. Remove the prehybridization solution and add 6 ml of the probe/DIG Easy Hyb mixture.

5. Incubate for 2 hours at +42°C.
   The hybridization solution with the DIG-labeled probe is stable at −15 to −25°C for >12 months and can be reused several times when freshly denatured at +65°C.
   Do not boil DIG Easy Hyb mixture.

Stringency washes after hybridization

1. Wash membrane two times in sufficient 2x SSC*, 0.1% SDS* (w/v) for 5 minutes each at +15 to +25°C.

2. Transfer to 0.5x SSC, 0.1% SDS (w/v) and wash twice for 15 minutes at +68°C with gentle agitation.

Chemiluminescent detection with CSPD

Perform all incubations at +15 to +25°C with shaking. The volumes of the solutions are calculated for one, 82 mm Ø disc. If more than one disc is processed in the same roller bottle, adjust the volumes accordingly to make sure that the membranes do not stick to each other and are sufficiently covered with solution.
See section, Working Solution for additional information on preparation of solutions.

1. After hybridization and post-hybridization washes, equilibrate the filter for approximately 1 to 5 minutes in Washing buffer.

2. Block the filter by incubating for 30 minutes in 40 ml Blocking solution.
   – For biotin-labeled probes, use 5% Blocking solution.

3. Incubate the membrane for 30 minutes in 15 ml Antibody solution.

4. Wash for 2 × 15 minutes in 40 ml Washing buffer.

5. Equilibrate for 2 to 5 minutes in 20 ml Detection buffer.

6. Incubate membrane for 5 minutes in 5 ml diluted CSPD* (0.25 mM) substrate solution.
   The diluted substrate solution can be reused 1 to 2 times.

7. Let excess liquid drip off and seal the damp membrane discs into a Hybridization Bag*.
   – Preincubate for 10 minutes at +37°C.
   Do not let the membranes dry to completion.

8. Expose to an imaging device or to X-ray film or Lumi-Film* for 15 to 25 minutes at +15 to +25°C.
   Keep membranes damp if stripping and reprobing is planned.

Handling high numbers of membranes

Use this protocol when numerous membranes are processed at the same time.

1. Place discs on a clean plastic sheet and add 1 ml substrate solution on top of each disc.
   Do not use Saran Wrap.

2. Distribute evenly by covering the membrane with a second sheet of plastic foil.

3. Incubate the covered membrane discs for 5 minutes at +15 to +25°C.

4. Gently squeeze excess liquid out off the sandwich and seal the plastic sheets.

5. Expose to an imaging device or to X-ray film or Lumi-Film* for 15 to 25 minutes at +15 to +25°C.

Probe removal

1. Wash membrane thoroughly in double-distilled water.

2. Incubate for 2 × 15 minutes at +37°C in 0.2 M NaOH, 0.1% SDS (w/v) with gentle agitation.

3. Equilibrate briefly in 2x SSC.
   – Air dry membrane or use directly for rehybridization.

Color detection with NBT/BCIP

Perform all incubations at +15 to +25°C with constant shaking except for the color reaction.
See section, Working Solution for additional information on preparation of solutions.

1. After hybridization and post-hybridization washes, equilibrate the filter for approximately 1 to 5 minutes in Washing buffer.

2. Block the filter by incubating for 30 minutes in 40 ml Blocking solution.
   – For biotin-labeled probes, use 5% Blocking solution.

3. Incubate the membrane for 30 minutes in 20 ml Antibody solution.

4. Wash for 2 × 15 minutes in 40 ml Washing buffer.

5. Equilibrate for 2 to 5 minutes in 20 ml Detection buffer.

6. Add 4 ml freshly prepared Color substrate solution to each membrane disc and incubate in the dark.
   ⚠ ***Process membranes separately. Do not shake or move during the incubation.***

7. When the expected signal appears, stop the reaction by rinsing in 50 ml TE buffer or double-distilled water.
   The color reaction is usually completed in 10 to 16 hours.

Documentation of results

1. Document the results by photocopying the wet filter or by photography.

2. Photocopying onto overhead transparencies allows for densitometric scanning.
   – Here, the color reaction can be interrupted for a short time and continued afterwards.

3. Store filters according to the following table:

IF...	THEN...
you want to reprobe your filters,	store filters in sealed plastic bags containing TE buffer. The color remains unchanged. ⚠ ***Do not let membranes dry out if they are to be reprobed.***
you do not want to reprobe your filters,	dry the filter at +15 to +25°C or bake at +80°C and store. *Color fades upon drying but can be revitalized by wetting the membrane with TE buffer.*

Stripping of color substrate

⚠ ***Always work under a fume hood.***

1. Heat a large beaker with dimethylformamide (DMF) to +50 to +60°C.
   ⚠ ***DMF is volatile and can be ignited at >+67°C.***

2. Incubate the membrane in the heated DMF until the blue color has been removed.
   Changing the DMF occasionally can speed the procedure. Extremely dense signals may be difficult to remove completely.

3. Rinse membrane thoroughly in double-distilled water.

4. Wash 2 × 20 minutes at +37°C in 0.2 M NaOH, 0.1% SDS* (w/v) to remove the DIG-labeled probe.

5. Rinse thoroughly in 2x SSC*.

6. Air dry membrane or use directly for hybridization.

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.
1 2 3 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.
Consumables		
Hybridization Bags	50 bags, 25 cm x 23 cm	11 666 649 001
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
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
DIG-High Prime	160 µl, 40 labeling assays	11 585 606 910
CSPD	1 ml	11 655 884 001
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
DIG Easy Hyb	500 ml	11 603 558 001
Blocking Reagent	custom fill	10 057 177 103
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
	100 films, 7.1 x 9.4 inches, 18 x 24 cm, *Not available in US*	11 666 916 001
DIG Easy Hyb Granules	6 bottles, Granules for 6 x 100 ml	11 796 895 001
Streptavidin-AP-conjugate for nucleic acid detection	150 U, (200 µl)	11 093 266 910
Biotin-High Prime	100 µl, 25 labeling assays	11 585 649 910
Fluorescein-High Prime	100 µl, 25 labeling assays	11 585 622 910
Proteinase K, recombinant, PCR Grade	850 mL	03 654 672 103
PCR DIG Probe Synthesis Kit	1 kit, 25 reactions of 50 µl final volume each. One reaction can produce enough labled probe to analyze 650 cm^2 of blot membrane.	11 636 090 910
Agarose MP	100 g	11 388 983 001
	500 g	11 388 991 001
Anti-Fluorescein, Fab fragments	Anti-Fluorescein-AP, Fab fragments, 150 U, 200 µl	11 426 338 910
	Anti-Fluorescein-POD, Fab fragments, 150 U	11 426 346 910
CDP-*Star*	1 ml	11 685 627 001
	2 x 1 ml	11 759 051 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001

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.

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