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APPLICATION GUIDE

Product:: TRANSFER-IT™ Plus MEMBRANE
Product No: TIP0303 *(23.2)*
Storage: Room Temperature

Membrane Description

TRANSFER-IT™ Plus is a nylon membrane that possesses pore surfaces populated by a high density of quaternary ammonium groups making it strongly cationic. The positive zeta potential is maintained over the range of pH 3 to over pH 10 and promotes strong ionic binding of negatively charged proteins and nucleic acids. TRANSFER-IT™ PLUS membrane is ideally suited to rapid transfer techniques for nucleic acids. In addition, the membrane's immobilization characteristics make it suitable for prolonged transfer procedures without the risk of nucleic acid diffusion from the membrane.

Membrane Handling

TRANSFER-IT™ PLUS membranes are intrinsically hydrophilic and do not require pre-wetting. The membranes are mechanically very strong and resistant to tearing or cracking, making removal from agarose or polyacrylamide gels particularly easy. TRANSFER-IT™ PLUS membranes should be handled using gloves or forceps to avoid membrane contamination. Either scissors or a sharp scalpel must be used to cut the membrane.

1. NUCLEIC ACID PROTOCOLS

1.1 SAMPLE PROTOCOL: DNA DOT BLOTS

The application of nucleic acid in solution directly onto a transfer membrane is termed a dot blot. DNA applied to TRANSFER-IT™ PLUS membrane should be fixed by baking at 80°C for 30 minutes, although UV cross-linking and microwave treatment also works well.

1.1.1 Denaturation of Target DNA:

Solubilize target DNA in 6X SSC (0.9 M sodium chloride; 90 mM sodium citrate). Denature solubilized DNA by heating to 95°C for 10 minutes followed by chilling on ice. Alternatively, DNA can be solubilized in 0.4 N NaOH, which will also cause denaturation.

1.1.2 Application of Spots to the Membrane:

Apply 0.1 pg to 1 ng of denatured target DNA in 1.0 - 1.5 µl total volume with a micro-pipettor to form discrete spots on the membrane. Larger volumes should be used if the spots are to be applied with a slot type blotting apparatus.

1.1.3 Rehydration:

Incubate membranes for 5 minutes with 0.5 ml/cm^2 of 2X SSC.

1.1.4 Hybridization and Detection

See Section 2

1.2 SOUTHERN TRANSFERS

Tests have shown that most detection systems yield best results when used with a modified alkaline transfer procedure. This procedure differs from the standard Southern transfer in that the gel is not neutralized after denaturation with high pH, high salt solution. This means that the initial stages of the transfer occur at alkaline pH, favoring attachment of the DNA to the membrane. The use of a neutral transfer buffer, slowly bringing the pH of the DNA back to 7.0, may aid in stabilizing the immobilized DNA on the membrane.

Excellent results can also be obtained using the original Southern transfer or alkaline transfer procedures (see *Molecular Cloning, A Laboratory Manual*, by Maniatis, Fritsch and Sambrook for detailed protocols).

1.2.1 Electrophorese digested DNA in an appropriate agarose gel to separate the DNA fragments. Use a sample buffer containing bromphenol blue.

1.2.2 Depurination:

It is often necessary to reduce the size of the DNA fragments in the gel after electrophoresis to facilitate transfer to a membrane. This is usually accomplished by incubating the gel in 2 volumes of 0.25 M HCl at room temperature. Incubation times should be limited to less than 20 minutes. Longer incubation times can render the DNA sequences unrecognizable to the probe.

1.2.3 Denaturation:

After depurination, the DNA strands must be separated before hybridization. The gel is rinsed in sterile water to remove excess HCl. The gel is then incubated in 2 volumes of Denaturation solution (0.5 M sodium hydroxide; 1.5 M sodium chloride) for 30 minutes (the bromphenol indicator dye should return to blue color; it does not, a second incubation step with Denaturation solution should be performed).

1.2.4 Alkaline Transfer of DNA:

This is a capillary transfer technique in which a dry transfer membrane is placed directly on the alkaline gel, over a wick saturated with neutral transfer buffer (20X SSC). To perform the transfer, the gel is transferred directly from the Denaturation solution to a buffer reservoir containing a supporting wick (generally 3MM Whatmann paper) and 20X SSC. A dry piece of TRANSFER-IT™ PLUS membrane is placed directly over the gel. This is covered with 1-3 sheets of Whatmann 1 paper, blotting pads, a top cover and a small weight (200-300 g).

NOTE: Blotting pads are recommended in place of paper towels that are sometimes used. The blotting pads ensure more even flow across the entire membrane area.

Transfer is generally allowed to proceed for 4-18 hours. Transfer of most fragments may be complete in as little as 2 hours. Minimum transfer times would have to be determined empirically for each type of DNA preparation. Alternatively, vacuum blotting or electro-transfer procedures can be used.

1.3 NORTHERN TRANSFER PROCEDURES

When working with RNA it is important to keep the equipment and reagent solutions free from RNase contamination. It is recommended that 0.1% SDS is incorporated in all solutions except the 20X SSC transfer buffer, and that all apparatus be cleaned with RNase-OFF™ (cat. #R0500).

1.3.1 Conditions for electrophoresis:

Electrophorese formamide denatured RNA in an appropriate formaldehyde agarose gel.

1.3.2 Capillary Transfer of RNA:

Use the procedure described in section 1.2.4 Gel should be neutralized before transfer by soaking in 2X SSC for 10 minutes.

NOTE: Overnight transfer is recommended for RNA to ensure complete recovery of higher molecular weight RNA from the gel. Other transfer procedures such as vacuum blotting or electro-transfer can be used but require extra equipment.

After transfer is complete remove the membrane from the gel surface. Do not rinse the membrane. Fix the RNA on the membrane. After fixation, proceed to hybridization and detection steps.

2. PREHYBRIDIZATION, HYBRIDIZATION AND VISUALIZATION

TRANSFER-IT™ PLUS membrane has been used successfully with many commercially available DNA detection kits. Manufacturer's recommendations should be followed with each kit. Additional information may be obtained from the kit manufacturer's technical support staff.

2.1 PREHYBRIDIZATION

Prehybridization and Hybridization procedures will vary with the type and source of probe DNA. Prehybridization (blocking non-specific binding sites on the membrane) is not required with all detection systems. Tests have shown that the most important components in a pre-hybridization solution are SDS and casein (BDH Hammersten cat #44020). Prehybridization can be performed for 30 minutes to several hours. In general, higher temperatures (up to 68°C) will result in faster and more efficient prehybridization.

2.2 HYBRIDIZATION

As with pre-hybridization, hybridization will vary according to the nature of probe DNA and the detection system used. Procedures routinely used for ^{32}P labeled probes are listed below:

2.2.1 The following solutions and procedures, standardized in many forensic labs, require no pre-hybridization step.

20.4	ml	sterile water
12.0	ml	50% PEG (mw 8000)
4.5	ml	20X SSPE
21.0	ml	20% SDS
57.9	ml	

Add probe and bring to a total volume of 60 ml with sterile water: Use a final concentration of 5 x 10^5 dpm/ml of labeled probe.

Add 0.1 ml/cm^2 of this hybridization solution to the membranes. Incubate at 65°C overnight with slow agitation.

2.2.2 Another commonly used procedure for hybridization is detailed below.

Heat denature an aliquot of non-homologous DNA.
Prepare hybridization solution:

100 µg/ml	non-homologous DNA
5X	SSC
5X	Denhardt's Solution
0.1% (w/v)	SDS

NOTE: Replacement of salmon sperm DNA and Denhardt's solution with 0.1% - 0.5% Hammersten grade casein may result in lower background.

2.2.2.1 Prehybridization:

Seal membrane in a plastic bag with 2 to 4 ml of hybridization solution per 100 cm^2 of membrane. Incubate at 65°C for Southerns and 42°C for Northerns for 30 minutes.

2.2.2.2 Hybridization:

Denature labeled probe by boiling for 5 minutes and snap cooling for 1 minute. Add sufficient denatured labeled probe to yield 2 ml of hybridization solution per 100 cm^2 of membrane. Remove the membrane from the prehybridization bag and place in a fresh bag with 2 ml/100 cm^2 of hybridization solution containing probe. Incubate at 65°C for Southerns and 42°C for Northerns for 4 hours to overnight.

2.3 POST HYBRIDIZATION WASHES

Place membranes in fresh bags or containers and wash with 2 ml/cm^2 as follows:

a. 15 minutes in 2X SSC, 0.1% SDS at room temperature
b. 15 minutes in 2X SSC, 0.1% SDS at room temperature
c. 20 minutes in 0.1X SSC, 0.1% SDS at 65°C (this solution should be preheated before use)
d. Remove excess liquid: Lightly blot the membrane on #1 Whatmann paper. Do not allow the membrane to dry.

2.4 AUTORADIOGRAPHY

Wrap the damp membranes in clear plastic. In a darkroom, assemble an autorad cassette with the side of the membrane that faced the gel against X-ray or autorad film (such as Kodak XAR). If intensifying screens are used, incubate film cassette at -80°C. Autorads should be exposed for 24 to 48 hours.

If membranes are to be reprobed, do not allow them to dry out at any stage following hybridization.

3. REHYBRIDIZATION

TRANSFER-IT™ PLUS membrane is easily reprobed. The procedure listed below is for use with both ^{32}P labeled and non-isotopic probes. In some systems which include protein in the prehybridization, hybridization or blocking steps, pretreatment with protease (0.5 mg/ml proteinase K, 0.1% SDS, at 65°C for 60 minutes) may be required to allow the stripping agents access to the hybridized DNA.

3.1 FORMAMIDE STRIPPING

WARNING: Formamide is a hazardous chemical. Health hazards include irritation on contact with skin, eyes or mucous membranes. Liver injury may occur. Ingestion leads to headache, dizziness, nausea, vomiting. Store formamide in a cool and dry area away from sources of fire. Use only inside fume hood, with chemical resistant gloves and safety pipette devices.
Incubate membranes in 20 ml/cm^2 of the following solution for 60 minutes at 65°C:

110	ml	formamide (filtered through XR-10 cation exchange resin)
20	ml	20X SSPE
10	ml	20% SDS
60	ml	sterile water

Rinse:
Rinse the stripped membrane in 20 ml/cm^2 of 0.1X SSC, 0.1% SDS for 1 minute at room temperature.

Remove excess liquid:
Place the membrane on filter paper for 30 seconds.
Rehybridize:
Repeat prehybridization and hybridization steps as for the original probe.

NOTE: If membranes are to be stored after stripping and rinsing, wrap the membranes with plastic wrap and freeze at -80°C.

3.2 BOILING 0.1% AQUEOUS SDS

Pour boiling 0.1% aqueous SDS solution onto the membrane and shake for a few minutes. Discard the solution and immediately add fresh boiling 0.1% SDS solution. Allow to cool to room temperature.

3.3 ALKALINE STRIPPING

This technique should not be used for RNA transfers. Add 0.4 M NaOH to the membrane. Incubate at 45°C for 30 minutes. The membrane is then washed with 0.1X SSC, 0.1% SDS for 15 minutes. This method of stripping is recommended for probes that will not strip under other conditions. *There may be some loss of target DNA with this protocol.*

3.4 FORMAMIDE (RNA TRANSFERS)

Add 75% Formamide (filtered through cation exchange resin) in 10 mM sodium phosphate buffer pH 7.5 to the wet membrane at 65°C for 1 hour. The membranes are washed with 0.1X SSC, 0.1% SDS for 15 minutes at room temperature. Check for probe removal and rehybridize as for the original probe.

TROUBLESHOOTING TIPS

The most common problems encountered in DNA detection are excessive or patchy backgrounds, and low sensitivity.

LOW SENSITIVITY

Low sensitivity in a system that has worked in the past:

- Low probe activity: Repeat experiment with freshly made probe.
- Incomplete probe denaturation: Ensure that the probe is properly heat denatured and snap cooled.
- Poor Transfer: Load extra lanes of control DNA and check transfer efficiency by staining the gel with ethidium bromide.
- Poor retention of DNA: Make sure that fixation procedures match recommendations. For instance, overexposure to UV light will result in a loss of sensitivity.

Low sensitivity in a system under development (***i.e.*** use of a newly available detection kit):

- Increased probe concentration
- Increase autorad (or color development) exposure time
- Decrease post-hybridization wash stringency
- Increase concentration of detection reagent-enzyme conjugate (for non-isotopic systems)

HIGH BACKGROUND

Background on DNA blots can be caused by non-specifically bound probe on the membrane, or by non-specifically bound enzyme conjugate (in non-isotopic systems). The following steps can be taken to decrease background:

- Ensure that the membrane stays fully wetted during pre-hybridization, hybridization and washing steps.
- Decrease probe concentration.
- Increase post hybridization wash stringency (raise temperature or reduce salt concentration)
- Increase blocking efficiency in pre-hybridization, hybridization and blocking (for non-isotopic systems) steps. Hammersten grade casein can be added to all these solutions, to a final concentration of 0.5%. Additionally, for non-isotopic systems, increasing the amount of SDS, up to 2% (w/v), in blocking buffers may be quite effective at increasing signal/noise.
- Increase final wash efficiency (non-isotopic systems)
- Decrease substrate exposure time
- Decrease substrate concentration

Non-specific background observed down the lanes of the blot:

Sometimes non-specific signal is seen in the lanes of the developed blot. This may be caused by

- Insufficient wash stringency
- Incomplete target DNA denaturation
- Genomic contamination of probe DNA
- Degradation of target DNA

REPROBING

Probe is not stripped from the membrane

- If membrane was dried after hybridization, it may not be possible to effectively remove probe DNA.
- Increase stringency of the stripping steps. Increase temperature, time of exposure to stripping agent, concentration of detergent and/or formamide.

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REFERENCES:

1. Dubitsky, A., Brown, J., and Brandwein, H. Chemiluminescent Detection of DNA on Nylon Membranes, *BioTechniques* 13 (1992) p. 392 - 399

2. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning. A laboratory manual. Cold Spring Harbor Laboratory, N.Y.

3. Meinkoth, J. and Wahl, G. Hybridization of nucleic acids immobilized on solid supports, *Anal. Biochem.* 138 (1984) p. 267 - 284

4. Nucleic Acid hybridization, a practical approach, (1985) Ed. Hames, B.D., and Higgins, S.J. IRL Press Ltd., Oxford, UK

5. Budowle, B. and Baechtel, F.S. Modifications to improve the effectiveness of restriction fragment length polymorphism typing. Appl. *Theor. Electrophor.* 1 (1990) p. 181 - 188

6. Reed, K.C. and Mann, D.A. Rapid transfer of DNA from agarose gels to nylon membranes. *Nucl. Acids Res.* 13 (1985) p. 7207 - 7221

7. Wahl, G.M., Stern, M. and Stark, G.R. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Acad. Sci.* USA 76 (1979) p 3686 - 3687