

ExpressHyb™ Hybridization Solution User Manual



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I. Introduction

Hybridization of nucleic acid probes to membrane-bound target sequences is a technique commonly used in gene structure and expression analysis, genome studies, and medical diagnostics. Success of this technique often depends on several factors that affect the rate of probe binding to target sequences. These factors include: hybridization temperature, concentration of probe, ionic strength, pH, and viscosity of hybridization solution (Wahl *et al.*, 1987).

ExpressHyb™ is a hybridization solution that has been optimized for Northern and Southern analyses on positively charged nylon membranes. Typical membrane hybridizations require long periods of incubation and involve conditions that are often difficult to optimize. The ExpressHyb protocol eliminates these difficulties by using a higher hybridization temperature and our specially formulated ExpressHyb Hybridization Solution. ExpressHyb requires only 1–2 hours of hybridization in contrast to the customary 12–24 hours. It also reduces background during nonradioactive detection, allowing detection of low-copy RNA species on Northern blots and single copy genes on Southern blots. ExpressHyb is also suitable for use with our Multiple Tissue Expression (MTE) Arrays.

ExpressHyb Solution can be used with DNA probes that are radioactively or nonradioactively labeled. Comparable results are obtained with both radioactive and chemiluminescent detection systems (using digoxigenin-labeled probes) after hybridization with ExpressHyb. Figure 1 illustrates the procedures for hybridization with either radioactively or nonradioactively labeled DNA probes.

I. Introduction *continued*

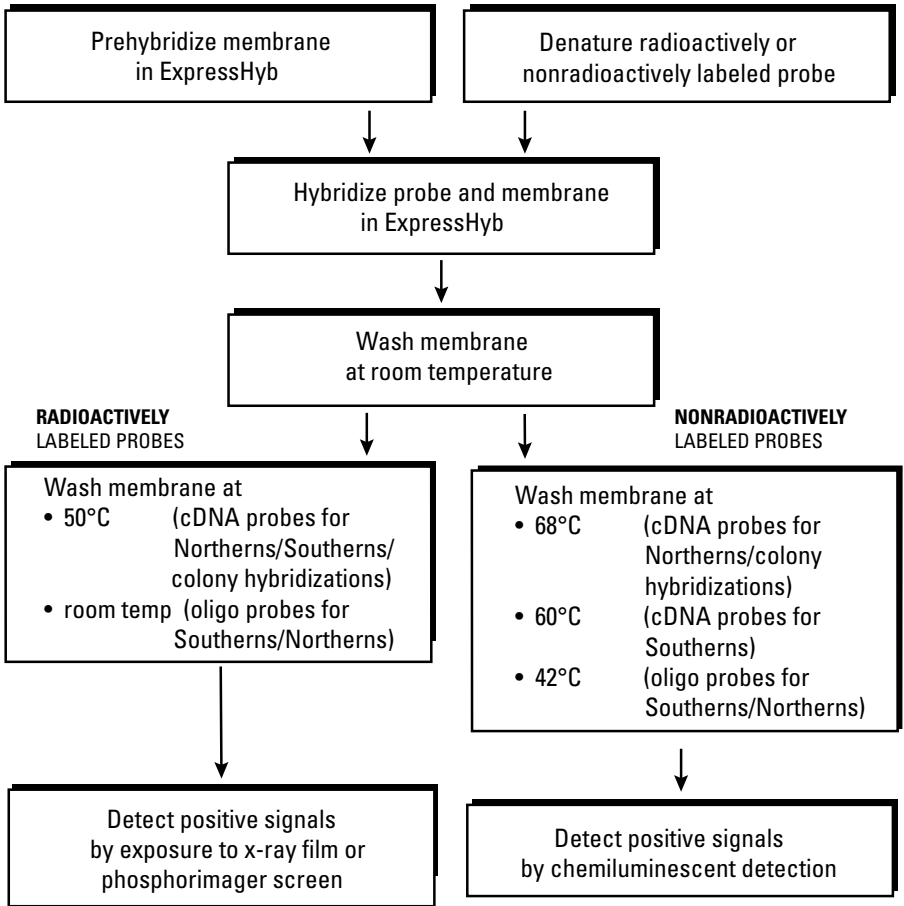


Figure 1. Flow chart of hybridization using ExpressHyb™ Hybridization Solution.

II. List of Components

Store ExpressHyb at room temperature.

- **ExpressHyb™ Hybridization Solution**

Catalog No. Amount

636831 250 ml

636832 500 ml

636833 1 L

III. Additional Materials Required

The following materials are required but not supplied:

- **20X SSC**

3 M NaCl

0.3 M Sodium citrate (pH 7.0)

- **Wash Solution 1**

2X SSC

0.05% SDS

- **Wash Solution 2**

0.1X SSC

0.1% SDS

- **Wash Solution 3**

2X SSC

0.1% SDS

IV. ExpressHyb™ Protocol

PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.

ExpressHyb Hybridization Solution should be stored at room temperature; however, at < 25°C, a precipitate may form. If this occurs, warm the solution and stir thoroughly to completely dissolve the precipitate. Avoid foaming by placing a stir-bar into the bottle and stirring slowly on a magnetic plate.

A. Hybridization with cDNA Probes

1. General considerations

- a. ExpressHyb Hybridization Solution can be used for Southern blot, Northern blot, or colony hybridizations. The only procedural difference between these three hybridizations is the incubation temperature.

Note: For Northern and colony hybridizations, incubate at 68°C. For Southern hybridizations, incubate at 60°C. Please see the MTE™ Array User Manuals for hybridization procedures specific for MTE™ products.

- b. The hybridization temperature used in the following protocols is suitable for hybridization of DNA probes of average GC content (40%). The optimal temperature for probes of different GC content must be determined empirically (Sambrook & Russell, 2001). To calculate the T_m based on the length of your probe, see the Appendix.
 - c. The recommended final DNA probe concentration is 2–10 ng/ml or $1-2 \times 10^6$ cpm/ml for Northern or Southern hybridizations (probe concentrations >10 ng/ml will reduce the time needed for hybridization, but may increase background). The recommended final DNA probe concentration for colony hybridizations is 100 ng/ml.
 - d. Concentrated probe should not be added directly to the membrane because uneven concentrations may result in anomalous signals.
- #### 2. Hybridization using ExpressHyb with **radioactively** labeled cDNA probes
- a. Warm the ExpressHyb Solution at 68°C (60°C, Southern blots), and stir well to completely dissolve any precipitate.
 - b. Prehybridize 10x 10-cm membranes in a minimum total volume of 5 ml of ExpressHyb Solution with continuous shaking at 68°C (60°C, Southern blots) for 30 min.
 - c. Denature the radioactively labeled DNA probe at 95–100°C for 2–5 min. Then chill quickly on ice.
 - d. Add radiolabeled probe to 5 ml of fresh ExpressHyb. Make sure that the probe is thoroughly mixed with the fresh ExpressHyb Solution.
 - e. Replace the ExpressHyb Solution with the fresh solution containing the radiolabeled DNA probe. Remove all air bubbles from the container, and make sure the ExpressHyb Solution is evenly distributed over the entire blot.

IV. ExpressHyb™ Protocol *continued*

- f. Incubate with continuous shaking at 68°C (60°C, Southern blots) for 1 hr.
 - g. Rinse the blot several times in Wash Solution 1 at room temperature. Wash for 30–40 min with continuous agitation; replace the wash solution several times.
 - h. Wash the blot in Wash Solution 2 with continuous shaking at 50°C (50°C, Southern blots) for 40 min with one change of fresh solution.
 - i. Remove the blot with forceps and shake off excess wash solution.
Note: Do not blot-dry the membrane. If the membrane is allowed to dry even partially, subsequent removal of the probe from the membrane may be difficult.
 - j. Immediately cover the blot with plastic wrap. Mount on Whatman paper (3 MM Chr). Wrap again with plastic wrap.
 - k. Expose to x-ray film at –70°C with two intensifying screens.
 - l. Remove the probe from the blot as outlined below.
 - i. Heat the sterile H₂O/0.5% SDS solution to 90–100°C.
 - ii. Remove plastic wrap from blot and immediately place in the heated solution. Make sure that exposure to air is minimal.
 - iii. Incubate for 10 min, shaking frequently.
 - iv. Allow the H₂O to cool for 10 min before removing the blot.
 - v. Remove the blot and air-dry until it is dry enough to be slipped into a plastic bag. The membrane can be stored at –20°C until needed.
3. Hybridization using ExpressHyb with **nonradioactively** labeled cDNA probes
- a. Warm the ExpressHyb Solution at 68°C (60°C, Southern blots), and stir well to completely dissolve any precipitate.
 - b. Prehybridize 10 x 10-cm membranes in a minimum total volume of 5 ml of ExpressHyb Solution with continuous shaking at 68°C (60°C, Southern blots) for 30 min.
 - c. Denature the nonradioactively labeled DNA probe at 95–100°C for 2–5 min. Then chill quickly on ice.
 - d. Add nonradiolabeled probe to 5 ml of fresh ExpressHyb. Mix thoroughly.
 - e. Replace the ExpressHyb Solution with the fresh solution containing the nonradiolabeled DNA probe. Remove all air bubbles from the container, and make sure the ExpressHyb Solution is evenly distributed over the entire blot.

IV. ExpressHyb™ Protocol *continued*

- f. Incubate with continuous shaking at 68°C (60°C, Southern blots) for 1 hr.
- g. Wash the membranes at room temperature for 30 min with at least 20 ml of Wash Solution 3 per 100-cm² membrane; replace the solution once.
- h. Wash the membranes in Wash Solution 2 at 68°C (60°C, Southern blots) for 30 min; replace the wash solution once.

Note: These washing conditions may be too stringent for probes which are not completely homologous to the target. If this is the case, lower the temperature to 50°C.

- i. Remove the blot with forceps, and shake off excess wash solution. Blots can then be used directly for chemiluminescent detection of hybridized DNA or can be stored air-dried for later detection using other nonradioactive detection systems, such as colorimetric systems.

Note: Do not blot-dry membrane. If the membrane is allowed to dry even partially, subsequent removal of the probe from the membrane may be difficult.

B. Hybridization with Oligonucleotide Probes

1. General considerations

- a. ExpressHyb Hybridization Solution can be used with oligonucleotide probes in Southern blot or Northern blot hybridizations.
- b. The hybridization temperature used in the following protocols is suitable for hybridization of oligonucleotide probes of average GC content (40%). The optimal temperature for probes of different GC content must be determined empirically (Sambrook *et al.*, 2001). To calculate the T_m based on the length of your probe, see the Appendix.
- c. The recommended final oligonucleotide probe concentration is 20–50 ng/ml or 1–2 x 10⁷ cpm/ml. Probe concentrations >50 ng/ml will reduce the time needed for hybridization, but may increase background.
- d. Concentrated probe should not be added directly to the membrane because uneven concentrations may result in anomalous signals.

2. Hybridization using ExpressHyb with **radioactively** labeled oligonucleotide probes

- a. Warm the ExpressHyb Solution at 68°C, and stir well to completely dissolve any precipitate. Then equilibrate the solution at 37°C.
- b. Prehybridize 10 x 10-cm membranes in a minimum of 5 ml of ExpressHyb Solution with continuous shaking at 37°C for 30 min.

IV. ExpressHyb™ Protocol *continued*

- c. Add radiolabeled probe to 5 ml of ExpressHyb. Make sure that the probe is thoroughly mixed with the fresh ExpressHyb Solution.
 - d. Replace the ExpressHyb Solution with the fresh solution containing the radiolabeled oligonucleotide probe. Remove all air bubbles from the container, and make sure the ExpressHyb Solution is evenly distributed over the entire blot.
 - e. Incubate with continuous shaking at 37°C for 1 hr.
 - f. Rinse the blot in Wash Solution 1 several times at room temperature. Wash for 30–40 min with continuous agitation; replace the wash solution several times.
 - g. Wash the blot in Wash Solution 2 with continuous shaking at room temperature for 40 min with one change of fresh solution.
 - h. Remove the blot with forceps and shake off excess wash solution.
Note: Do not blot-dry membrane. If the membrane is allowed to dry even partially, subsequent removal of the probe from the membrane may be difficult.
 - i. Immediately cover the blot with plastic wrap. Mount on Whatman paper (3MM Chr). Wrap again with plastic wrap.
 - j. Expose to x-ray film at –70°C with two intensifying screens.
 - k. Remove the probe from the blot as outlined below.
 - i. Heat the sterile H₂O/0.5% SDS solution to 90–100°C.
 - ii. Remove plastic wrap from blot and immediately place in the heated solution. Make sure that exposure to air is minimal.
 - iii. Incubate for 10 min, shaking frequently.
 - iv. Allow the H₂O to cool for 10 min before removing the blot.
 - v. Remove the blot and air-dry until it is dry enough to be slipped into a plastic bag. The membrane can be stored at –20°C until needed.
3. Hybridization using ExpressHyb with **nonradioactively** labeled oligonucleotide probes
- a. Warm the ExpressHyb Solution at 68°C, and stir well to completely dissolve any precipitate. Then equilibrate the solution at 42°C.
 - b. Prehybridize 10 x 10-cm membranes in a minimum of 5 ml of ExpressHyb Solution with continuous shaking at 42°C for 30 min.
 - c. Add nonradiolabeled probe to 5 ml of ExpressHyb. Make sure that the probe is thoroughly mixed with the fresh ExpressHyb Solution.
 - d. Replace the ExpressHyb Solution with the fresh solution containing the nonradiolabeled oligonucleotide probe. Remove all air bubbles from the container, and make sure the ExpressHyb Solution is evenly distributed over the entire blot.

IV. ExpressHyb™ Protocol *continued*

- e. Incubate with continuous shaking at 42°C for 1 hr.
- f. Wash the membranes at room temperature for 30 min with at least 20 ml of Wash Solution 3 per 100-cm² membrane; replace the solution once.
- g. Wash the membranes in Wash Solution 2 at 42°C for 30 min; replace the wash solution once.
- h. Remove the blot with forceps, and shake off excess wash solution. Blots can then be used directly for chemiluminescent detection of hybridized DNA or can be stored air-dried for later detection using other nonradioactive detection systems, such as colorimetric systems.

Note: Do not blot-dry membrane. If the membrane is allowed to dry even partially, subsequent removal of the probe from the membrane may be difficult.

V. Troubleshooting Guide

A. High background (with or without hybridization signals)

Concentration of the probe is too high. Do not exceed 2×10^6 cpm/ml for radioactively labeled probes. Do not exceed 30 ng/ml for nonradioactively labeled probes.

Length of DNA probe is too long. The optimal DNA probe length is 200–800 nucleotides.

Hybridization time is too long. Reduce hybridization time from 1 hr to 30 min.

B. Hybridization signals absent or very weak

General considerations. Make sure that ExpressHyb Hybridization Solution is distributed evenly over the entire blot.

Do not add the radioactively or nonradioactively labeled probe directly to the blot. Mix the probe with ExpressHyb Hybridization Solution before adding to the blot.

Continuously shake the blot during hybridization. Remove any air bubbles from the blot during hybridization and when the blot is wrapped in plastic.

Do not allow the blot to dry at any step during hybridization or detection.

Specific activity of radioactively labeled probe is too low. Specific activity of the probe should be $>5 \times 10^8$ cpm/ μ g (Sambrook & Russell, 2001). If your probe's specific activity is lower, make a new probe with fresh 32 P.

Too little DNA being used in the hybridization. Typically 25–50 ng of labeled probe is used in each hybridization. Determine the amount of probe being used by visually comparing the signal intensities of the probe and a known amount of DNA markers on an ethidium bromide-stained agarose gel. If a clear ethidium bromide-stained band is not observed for the probe, less than 25–50 ng is being used. If this is the case, use 2–3 times more DNA for probe labeling.

VI. References

Sambrook, J. & Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Wahl, G. M., Berger, S. L. & Kimmel, A. R. (1987) Molecular hybridization of immobilized nucleic acids: Theoretical concepts and practical considerations. *Methods Enzymol.* **152**:399–407.

Appendix: Calculation of T_m

For DNA molecules longer than 200 nucleotides, perform the hybridization at 15–25°C below the calculated melting temperature (T_m) of a perfect hybrid. Use the following equation to calculate the T_m based on the length of your probe.

$$T_m = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41\left[\frac{(\text{G}+\text{C})}{n}(100)\right] - \frac{600}{n}$$

where n = number of nucleotides and the concentration of Na^+ is 1.0 M or less.

Note: The Na^+ concentration in ExpressHyb is 0.5 M.