

Clontech Laboratories, Inc.

ExpressHyb™ Hybridization Solution User Manual

Cat. No(s). 636831, 636832 & 636833
(111511)

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I. Introduction

A. Summary

ExpressHyb (Cat. Nos. 636831, 636832 & 636833) is a hybridization solution that provides faster hybridizations with increased sensitivity. It is ideal for cDNA array, Northern and Southern blot, and colony hybridization applications—and can be used with DNA probes that are radioactively or nonradioactively labeled.

B. Major Advantages over Other Hybridization Methods

Typical membrane hybridizations require long periods of incubation and involve conditions that are often difficult to optimize, since they depend 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). The ExpressHyb protocol eliminates these difficulties by using a higher hybridization temperature and our specially formulated ExpressHyb Hybridization Solution, providing the following advantages over other hybridization methods:

- Requires only 1–2 hours of hybridization instead of the usual 12–24 hours.
- Reduces background during nonradioactive detection, allowing detection of low-copy RNA species on Northern blots and single copy genes on Southern blots.
- Comparable results are obtained with both radioactive and chemiluminescent detection systems (using digoxigenin-labeled probes).

Figure 1 shows hybridization procedures using either radioactively or nonradioactively labeled DNA probes.

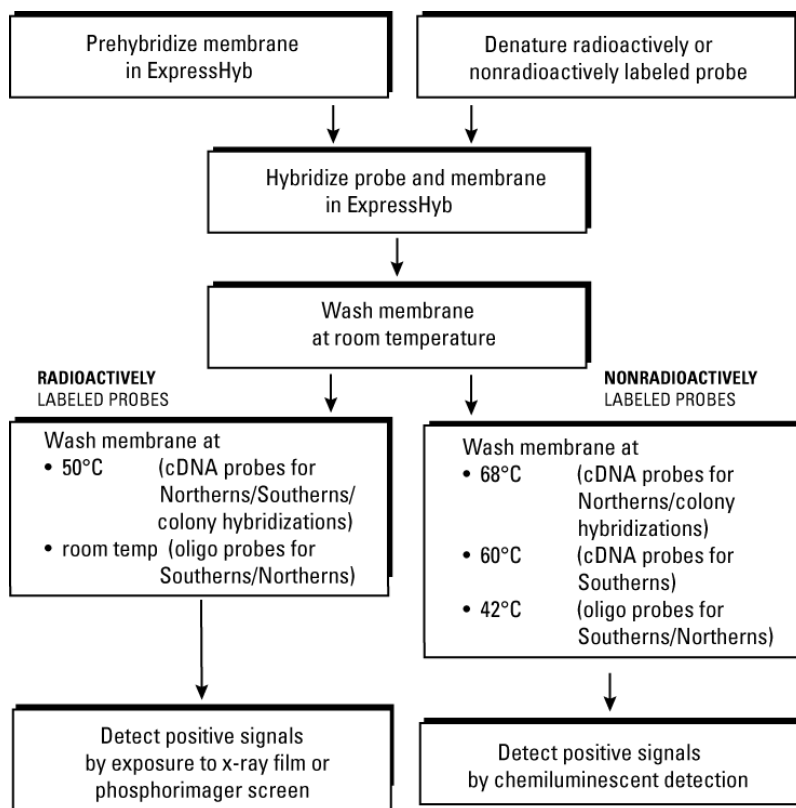


Figure 1. Flow chart of hybridization using ExpressHyb Hybridization Solution.

II. List of Components

ExpressHyb Hybridization Solution

Store ExpressHyb at room temperature.

<u>Cat. No.</u>	<u>Amount</u>
636831	250 ml
636832	500 ml
636833	1 L

III. Additional Materials Required

The following reagents 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. General Considerations

A. Storage Conditions

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

B. Determining the Optimal Hybridization Temperature

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. Adding Probe to Membranes

Concentrated probe should not be added directly to the membrane because uneven concentrations may result in anomalous signals. Mix probe with the specified volume of ExpressHyb first (see Section V).

D. cDNA Probe-Specific Applications & Conditions

1. **Applications:** Southern blot, Northern blot, or colony hybridization procedures, which differ only with respect to the incubation temperature.

NOTE: Incubate Northern and colony hybridizations at 68°C, and Southern hybridizations at 60°C, using either radioactive or nonradioactive probes.

2. **Recommended final DNA probe concentration:** 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.

E. Oligonucleotide Probe-Specific Applications & Conditions

1. **Applications:** Southern blot or Northern blot hybridizations, which are incubated at the same temperature.

NOTE: Incubate Northern and Southern hybridizations at 37°C, using radioactive probes, and 42°C using nonradioactive probes.

2. **Recommended final oligonucleotide probe concentration:** 20–50 ng/ml or $1-2 \times 10^7$ cpm/ml. Probe concentrations >50 ng/ml will reduce the time needed for hybridization, but may increase background.

V. ExpressHyb Protocols

Unless specified in **bold**, conditions are the same for cDNA and oligonucleotide probes.

A. Hybridization Using Radioactively Labeled cDNA & Oligonucleotide Probes

1. Warm the ExpressHyb Solution at 68°C (60°C, Southern blots), and stir well to completely dissolve any precipitate. **Then (for oligonucleotide probes only) equilibrate the solution at 37°C.**
2. Prehybridize 10 x 10-cm membranes in a minimum total volume of 5 ml of ExpressHyb Solution with continuous shaking for 30 min **at 68°C (60°C, Southern blots) using cDNA probes, or 37°C using oligonucleotide probes.**
3. **Denature (cDNA probes only) at 95–100°C for 2–5 min. Then chill quickly on ice.**
4. Add probe to 5 ml of fresh ExpressHyb and mix thoroughly.
5. Replace the ExpressHyb Solution with the fresh solution containing the radiolabeled probe. Remove all air bubbles from the container, and make sure the ExpressHyb Solution is evenly distributed over the entire blot.
6. Incubate with continuous shaking for 1 hr at **68°C (60°C, Southern blots) using cDNA probes, or 37°C using oligonucleotide probes.**
7. 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.
8. Wash the blot in Wash Solution 2 with continuous shaking for 40 min **at 50°C (50°C, Southern blots) using cDNA probes, or room temperature using oligonucleotide probes**, with one change of fresh solution.
9. Remove the blot with forceps and shake off excess wash solution.

IMPORTANT: 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.

10. Immediately cover the blot with plastic wrap. Mount on Whatman paper (3 MM Chr). Wrap again with plastic wrap.
11. Expose to x-ray film at –70°C with two intensifying screens.
12. Remove the probe from the blot as outlined below.
 - a. Heat the sterile H₂O/0.5% SDS solution to 90–100°C.
 - b. Remove plastic wrap from blot and immediately place in the heated solution. Make sure that exposure to air is minimal.
 - c. Incubate for 10 min, shaking frequently.
 - d. Allow the H₂O to cool for 10 min before removing the blot.
 - e. 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.

B. Hybridization Using Nonradioactively Labeled cDNA & Oligonucleotide Probes

1. Warm the ExpressHyb Solution at **68°C (60°C, Southern blots) using cDNA probes, or 68°C using oligonucleotide probes** and stir well to completely dissolve any precipitate. **Then (for oligonucleotide probes only) equilibrate the solution at 42°C.**
2. Prehybridize 10 x 10-cm membranes in a minimum total volume of 5 ml of ExpressHyb Solution with continuous shaking for 30 min at **68°C (60°C, Southern blots) using cDNA probes, or 42°C using oligonucleotide probes.**
3. **Denature (cDNA probes only) at 95–100°C for 2–5 min. Then chill quickly on ice.**
4. Add nonradiolabeled probe to 5 ml of fresh ExpressHyb and mix thoroughly.
5. Replace the ExpressHyb Solution with the fresh solution containing the nonradiolabeled probe. Remove all air bubbles from the container, and make sure the ExpressHyb Solution is evenly distributed over the entire blot.
6. Incubate with continuous shaking for 1 hr at **68°C (60°C, Southern blots) using cDNA probes, or 42°C using oligonucleotide probes.**
7. 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.
8. Wash the membranes in Wash Solution 2 for 30 min at **68°C (60°C, Southern blots) using cDNA probes, or 42°C using oligonucleotide probes;** replace the wash solution once.

NOTE (for cDNA probes only): 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.

9. 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.

IMPORTANT: 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.

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.

VII. Troubleshooting Guide

A. High background (with or without hybridization signals)

Description of Problem	Solution
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 the 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

Description of Problem	Solution
General considerations	<ul style="list-style-type: none"> • 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 it 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 was 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.

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.

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

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.

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