# QuikHyb Hybridization Solution

## INSTRUCTION MANUAL

Catalog #201220 (250 ml) and #201221 (1 liter)
Revision B.0

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201220-12

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# QuikHyb Hybridization Solution

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# QuikHyb Hybridization Solution

## MATERIALS PROVIDED

| Material provided | Quantity | |
|---|---|---|
| | **Catalog #201220** | **Catalog #201221** |
| QuikHyb hybridization solution[a] | One 250-ml bottle | One 1-liter bottle |

[a] Store at 4°C. Do not freeze.

***Note*** *The molar concentration of NaCl in QuikHyb hybridization solution is 0.5 M.*

## STORAGE CONDITIONS

**QuikHyb hybridization solution:** 4°C

Revision B.0

# INTRODUCTION

Nucleic acid hybridization of a nucleic acid probe to a target sequence immobilized on a solid support such as a nylon membrane is a powerful technique routinely used in the analysis of gene structure and expression and in the diagnosis of disease.

Factors that affect the rate and efficiency of nucleic acid binding to a target sequence include hybridization time, temperature, concentration of the target sequence and concentration of the probe. Other variables which improve the hybridization reaction are the use of rate enhancers and blocking agents to suppress nonspecific hybridization.

QuikHyb hybridization solution* was developed for use in Southern, Northern, slot-blot and plaque-lift formats using randomly labeled radioactive and nonradioactive nucleic acid probes. This easy-to-use solution reduces the time required for hybridization from the customary 12–24 hours down to 1–2 hours, resulting in sensitive detection of target sequences.

# RADIOACTIVE HYBRIDIZATION WITH QUIKHYB HYBRIDIZATION SOLUTION

## Prehybridization

***Notes*** *Gently invert the QuikHyb hybridization solution immediately before use in order to ensure a homogenous mixture.*

*Prior to prehybridization (after crosslinking or baking), briefly dip the membrane in deionized water to remove excess salt from the membrane.*

*The membrane should be covered with a thin film of the QuikHyb solution at all times.*

1. Use a minimum of 33 µl of the QuikHyb hybridization solution/cm$^2$ of the blot. If this volume is not sufficient to cover the membrane at all times during the prehybridization and hybridization, add more solution until it does.

### Minimum Total Volume

2 ml when using 50-ml conical tubes
10 ml when using heat-sealable bags or roller bottles

* Patent Pending.

2. For *double-stranded probes*, prehybridize the membrane in QuikHyb solution at 68°C for 10–20 minutes.

   For *oligonucleotide probes and riboprobes*, calculate the melting temperature ($T_m$) (see *Appendix I: Hybridization and Melting Temperatures* for the mathematical formula). Prehybridize the membrane in QuikHyb solution for 10–20 minutes at 5–10°C below the $T_m$.

## Probe Preparation and Hybridization

Add the labeled probe to 100 µl of 10 mg/ml sonicated salmon sperm DNA.

### Suggested Probe Concentration

$2.5 \times 10^6$ total counts/2 ml of hybridization solution

### Specific Activity of the Probe

$10^8$ cpm/µg or greater

### Double-Stranded Probes

Boil the double-stranded probe and salmon sperm DNA mixture for 2 minutes and then add the mixture to the prehybridization solution. Due to the viscosity of the QuikHyb hybridization solution, the probe may not mix well in the large glass tubes (roller bottles) used in hybridization ovens. Perform the following steps to ensure the probe is mixed well with the prehybridization solution.

1. Remove 1 ml of the prehybridization solution from the glass tube and mix the solution with the probe.

2. Place this mixture back in the glass tube and hybridize in QuikHyb solution at 68°C for 1 hour.

### Oligonucleotide Probes and Riboprobes

Add the probe and salmon sperm DNA mixture directly to the prehybridization solution without boiling. Due to the viscosity of the QuikHyb hybridization solution, the probe may not mix well in the large glass tubes (roller bottles) used in hybridization ovens. Perform the following steps to ensure the probe is mixed well with the prehybridization solution.

1. Remove 1 ml of the prehybridization solution from the glass tube and mix the solution with the probe.

2. Place this mixture back in the glass tube and hybridize in QuikHyb solution at 5–10°C below the $T_m$ for 1 hour (see *Appendix I: Hybridization and Melting Temperatures*).

## Washing the Membrane

Perform the following washes (gentle agitation is required) for the double-stranded probes, oligonucleotide probes and riboprobes:

1. Wash twice for 15 minutes at room temperature with a 2× SSC buffer and 0.1% (w/v) SDS wash solution[§] (~2.5 ml/cm$^2$).

2. Wash once for 30 minutes at 60°C with a 0.1× SSC buffer and 0.1% (w/v) SDS wash solution[§] for a high-stringency wash.

## Detection

Wrap the membrane in plastic wrap and place the wrapped membrane on Kodak® X-OMAT® AR film with an intensifying screen at –80°C. Expose the film overnight.

## Stripping the Membrane for Reuse

1. Heat the 0.1× SSC buffer and 0.1% (w/v) SDS wash solution to boiling.

2. In a glass dish, pour the 0.1× SSC buffer and 0.1% (w/v) SDS wash solution over the membrane and wash the membrane twice for 15 minutes.

3. Proceed with the prehybridization step for the next hybridization or store the blot in plastic wrap, desiccated.

[§] See *Preparation of Media and Reagents.*

# APPENDIX I: HYBRIDIZATION AND MELTING TEMPERATURES

## Calculating the Hybridization Temperature

The hybridization temperature for oligonucleotide probes equals 5–10°C below the $T_m$. For best results with oligonucleotide probes, perform hybridizations for 1 hour.

## Calculating the Melting Temperature[1]

***Notes*** *The first method below overestimates the Tm of hybrids involving longer nucleotides. The second formula works only for Na+ concentrations of ≤1 M.*

*The molar concentration of NaCl in QuikHyb hybridization solution is 0.5 M.*

### Oligonucleotides Shorter than 18 Bases

$T_m = 2°C(A + T) + 4°C(G + C)$

### Oligonucleotides 14 Bases and Longer (up to 60–70 Nucleotides)

$T_m = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) – (600/N)$

where $N$ is the chain length.

## PREPARATION OF MEDIA AND REAGENTS

| | |
|---|---|
| **20× SSC Stock Solution**<br>175.3 g of NaCl (3 M final concentration)<br>88.2 g of sodium citrate-trisodium salt (300 mM final concentration)<br>Adjust the pH to 7.0 with HCl<br>Add distilled water ($dH_2O$) to 1 liter | **20% (w/v) SDS Stock Solution**<br>Dissolve 20 g of SDS in 90 ml of distilled water<br>Mix well and heat to 68°C if necessary<br>Then add $dH_2O$ to a final volume of 100 ml |
| **2× SSC Buffer and 0.1% (w/v) SDS Wash Solution**<br>100 ml of 20× SSC buffer<br>5 ml of 20% (w/v) SDS<br>$dH_2O$ to 1 liter | **0.1× SSC Buffer and 0.1% (w/v) SDS Wash Solution**<br>5 ml of 20× SSC buffer<br>5 ml of 20% (w/v) SDS<br>$dH_2O$ to 1 liter |

## REFERENCE


1. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.


## ENDNOTES



## MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

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# QuikHyb Hybridization Solution

Catalog #201220 (250 ml) and #201221 (1 liter)

## QUICK-REFERENCE PROTOCOL

### Radioactive Hybridization

- Prehybridize for 15 minutes
- Denature the double-stranded probe
- Add probe to the prehybridizing blot
- Hybridize for 1 hour
- Wash twice for 15 minutes at room temperature with a 2× SSC buffer and 0.1% (w/v) SDS wash solution
- Wash once for 30 minutes at higher stringency temperature with a 0.1× SSC buffer and 0.1% (w/v) SDS wash solution
- Expose to Kodak® X-OMAT® AR film at –80°C overnight