Miracle*Hyb* Hybridization Solution

INSTRUCTION MANUAL

Catalog #201223 (125 ml) and 201222 (250 ml)
Revision B.0

For Research Use Only. Not for use in diagnostic procedures
201222-12

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada

Agilent Technologies
Stratagene Products Division
11011 North Torrey Pines Road
La Jolla, CA 92037
Telephone (858) 373-6300
Order Toll Free (800) 424-5444
Technical Services (800) 894-1304
Internet techservices@agilent.com
World Wide Web www.stratagene.com

Europe

Location	Telephone	Fax	Technical Services
Austria	0800 292 499	0800 292 496	0800 292 498
Belgium	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 15775	0800 15740	0800 15720
France	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 919 288	0800 919 287	0800 919 289
Germany	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 182 8232	0800 182 8231	0800 182 8234
Netherlands	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 023 0446	+31 (0)20 312 5700	0800 023 0448
Switzerland	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 563 080	0800 563 082	0800 563 081
United Kingdom	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 917 3282	0800 917 3283	0800 917 3281

All Other Countries

Please contact your local distributor. A complete list of distributors is available at www.stratagene.com.

MIRACLE*HYB* HYBRIDIZATION SOLUTION

CONTENTS

Miracle*Hyb* Hybridization Solution

MATERIALS PROVIDED

Materials provided	Quantity	
	Catalog number 201223	**Catalog number 201222**
Miracle*Hyb* probe preparation buffer	2 x 1 ml vials	3 x 1 ml vials
Miracle*Hyb* hybridization solution	125 ml	250 ml

STORAGE CONDITIONS

Miracle*Hyb* Hybridization Solution: Room Temperature
Miracle*Hyb* Probe Preparation Buffer: +4°C

ADDITIONAL MATERIALS REQUIRED

deionized H_2O
hybridization containers
SSC/SDS wash solutions (see *Preparation of Media and Reagents*)
Film for autoradiography

Revision B.0

INTRODUCTION

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

Miracle*Hyb* hybridization solution was developed for use in Northern analysis when sensitive detection of low-abundance messages is a priority. In typical Northern applications, high complexity RNA samples are size-fractionated by agarose gel electrophoresis, then the resolved RNAs are transferred by blotting to a solid support. The immobilized RNAs may then be interrogated with one or more gene-specific probes. Miracle*Hyb* is useful for hybridization experiments to detect both low- and high-abundance RNAs. When detecting low-abundance transcripts or when high signal intensity is required, a 24-hour hybridization is performed to achieve maximum sensitivity without increasing background levels. When studying high-abundance genes, or when quick results are important, hybridization time may be reduced to 1–2 hours. Miracle*Hyb* can also be used in Southern and slot-blot formats.

Miracle*Hyb* is stored at room temperature and dispensed directly from the bottle. Heating or mixing is not required prior to prehybridization/ hybridization.

RADIOACTIVE HYBRIDIZATION WITH MIRACLE*HYB* HYBRIDIZATION SOLUTION

Immobilization of target sequences on membranes prior to hybridization may be accomplished using a variety of protocols. See References 1 and *2* for standard blotting protocols.

Notes *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 Miracle*Hyb *hybridization solution at all times.* ***Once wetted, do not allow the membrane to dry out at any point in the procedure.***

1. Generally, 180 µl of the Miracle*Hyb* hybridization solution per cm^2 of the blot should be used for standard experiments. More or less Miracle*Hyb* hybridization solution may be used as desired, but it is necessary to ensure that there is sufficient solution to cover the membrane at all times during the prehybridization and hybridization. The table below gives recommended minimum volumes of Miracle*Hyb* hybridization solution for common hybridization containers.

Container	Minimum volume of Miracle*Hyb* hybridization solution
50-ml conical tubes	5 ml
heat-sealable bags or roller bottles	10 ml

2. For **double-stranded probes**, prehybridize the membrane in Miracle*Hyb* hybridization solution at 68°C for 15 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 Miracle*Hyb* solution for 15 minutes at 5–10°C below the T_m.

   Note *It is normal for the Miracle*Hyb *hybridization solution to become slightly opaque during the pre-hybridization step, especially at higher incubation temperatures (≥50°C).*

3. Aliquot into a screw-cap microcentrifuge the correct amount of Miracle*Hyb* probe preparation buffer (50 µl of Miracle*Hyb* probe preparation buffer per 5 ml of prehybridization solution used in Step 2.)

   Note *Always use screw-cap microcentrifuge tubes when boiling radioactive solutions*

4. Add radiolabeled probe to the Miracle*Hyb* probe preparation buffer and mix by pipetting. For double-stranded probes, boil the diluted probe for 2 minutes. Briefly spin in a bench top microcentrifuge. **The boiling step is not necessary for oligonucleotide or RNA probes.**

   For best results, use random-primed radioactive probes with the following concentration and specific activity:

   Suggested Probe Concentration
   1.0×10^6 total counts/ml of hybridization solution

   Specific Activity of the Probe
   10^8 cpm/µg or greater

5. Add the probe/Miracle*Hyb* probe preparation buffer mixture to the prehybridization solution containing the blot. **It is important that the probe is added to the prehybridization solution and is not pipetted directly onto the membrane.**

Hybridization Conditions

Carry out the hybridization at 68°C. (When using oligonucleotide probes or other short probes, perform the hybridization at 5–10°C below the T_m.) Probe may be hybridized to the blot (on roller bars or a similar device for gentle agitation) for as few as one to two hours for high-abundance messages or standard Southern blot analysis. For low-abundance messages or when maximum sensitivity is desired, hybridize overnight (≥16 hours).

Washing the Membrane

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

1. Wash twice for 15 minutes each at room temperature with excess 2× SSC buffer and 0.1% (w/v) SDS wash solution[§].

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

Detection

Using forceps, grasp the membrane by one corner and lift it out of the wash solution. Hold it vertically so that the excess liquid is allowed to drain off. Remove the last traces of excess liquid by touching the bottom corner of the membrane to a clean paper towel or tissue. Wrap the membrane in plastic wrap. Expose the wrapped membrane to autoradiography film with an intensifying screen at –80°C for 2 hours–overnight. For very low-abundance messages or increased signal intensity, film exposure may be extended up to one week without greatly increasing background.

Stripping the Membranes 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 each.

Proceed with the prehybridization step for the next hybridization. If the blot will not be used immediately for a second round of hybridization, remove the excess liquid from the membrane by draining (see *Detection* above), then store the membrane in plastic wrap, desiccated.

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

APPENDIX I: HYBRIDIZATION AND MELTING TEMPERATURES

Hybridization Temperature

The hybridization temperature for oligonucleotide probes equals 5–10°C below the T_m.

Calculation of the Melting Temperature

Note *The first equation below overestimates the* T_m *of hybrids involving longer oligonucleotides. The second formula works only for monovalent cation concentrations of ≤1 M. The molar concentration of monovalent cations in Miracle*Hyb *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)

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

where [*MC*] is the molar concentration of monovalent cations and *N* is the chain length.

More discussion regarding calculation of melting temperature can be found in references 1 and 2.

PREPARATION OF MEDIA AND REAGENTS

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

REFERENCES

1. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G. *et al.* (1987). *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.
2. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

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.

STRATAGENE

An Agilent Technologies Division

Miracle*Hyb*® Hybridization Solution

Catalog #201223 (125 ml) and #201222 (250 ml)

QUICK-REFERENCE PROTOCOL

Radioactive Hybridization

- Prehybridize the blot in Miracle*Hyb* hybridization solution for 15 minutes at 68°C for double-stranded probes (or 5–10°C below the T_m for oligonucleotide probes)
- Aliquot into a microcentrifuge the correct amount of Miracle*Hyb* probe preparation buffer (50 µl of Miracle*Hyb* probe preparation buffer per 5 ml of prehybridization solution used.)
- Add radiolabeled probe to the Miracle*Hyb* probe preparation buffer and mix by pipetting. For double-stranded probes, boil the diluted probe for 2 minutes. Briefly spin in a bench top microcentrifuge. The boiling step is not necessary for oligonucleotide or RNA probes.
- Add the probe/Miracle*Hyb* probe preparation buffer mixture to the prehybridization solution containing the blot
- Hybridize at 68°C (or 5–10°C below the T_m for oligonucleotide probes) for 1 hour to overnight
- Wash twice for 15 minutes each at room temperature with 2× SSC buffer and 0.1% (w/v) SDS wash solution
- Wash once for 30 minutes at 60°C with 0.1× SSC buffer and 0.1% (w/v) SDS wash solution
- Expose to film at –80°C for 2 hours to overnight