ambion
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NorthernMax® Prehybridization/Hybridization Buffer

Catalog Number AM8677

Pub. No. 4386620 **Rev.** B

Contents	Quantity	Storage conditions
NorthernMax® Prehybridization/Hybridization Buffer	500 mL	Store at 4°C.

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product Description

NorthernMax® Prehybridization/Hybridization Buffer is a prehybridization/hybridization buffer with proprietary blocking and background reduction agents.

Appearance: Clear, light yellow solution.

Use NorthernMax® Prehybridization/Hybridization Buffer

Note: In this procedure, shaking is not critical as long as the membrane is level and is evenly immersed in buffer at all times. Multiple membranes may be treated within the same vessel as long as there is sufficient buffer to keep membranes from sticking together.

1. Warm 10 mL of Prehybridization/Hybridization Buffer per 100 cm^2 of membrane at the desired hybridization temperature (see step 5) for 15 minutes before use.
2. Prehybridize by placing the membrane in the Prehybridization/Hybridization Buffer for 30 minutes at approximately 65°C for RNA probes and 42°C for DNA probes.
3. For DNA probes prepared by nick translation, random priming, or PCR, denature prior to use by boiling in a small volume of the hybridization solution for 5 minutes.
4. Following prehybridization, combine enough of the boiled probe to the prehybridization solution to bring the probe to the proper final concentration.
   Generally, radioactive probe concentrations should be 1–5 x 10^6 cpm/mL hybridization buffer, and non-radioisotopically labeled probes should be 0.1 nM (10–20 ng/mL).
5. Hybridize overnight (8–16 hours) at the appropriate temperature (usually 10–25°C below the calculated T_m) with constant agitation.

T_m calculation based on probe type

Probe type	T_m Calculation
RNA probe	T_m = 53°C + 0.7(%G + C) – 500/duplex length – 1°C per %mismatch
DNA probe	T_m = 35°C + 0.8(%G + C) – 500/duplex length – 1°C per %mismatch
Oligonucleotide probe	T_m = 35°C + 0.8(%G + C) – 500/duplex length – 1°C to 1.5°C per %mismatch

Pre-hybridization and hybridization temperature by probe type

Probe type	Prehyb/hyb temp
DNA probes larger than ~50 bp [1]	42°C
RNA probes larger than ~50 bases	68°C
Oligonucleotide probes up to ~50 bases[2]	37°C to 42C°

[1] DNA probes prepared by random-primed labeling will be on average about half the size of the template used in the labeling reaction.
[2] Use a 37°C hybridization temperature initially, and raise the temperature if cross-hybridization is seen.

Quality Control

Nonspecific endonuclease activity: A sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity: A sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

RNase activity: A sample is incubated with labeled RNA and analyzed by PAGE.

Functional Testing: NorthernMax® Prehybridization/Hybridization Buffer is functionally tested using the Ambion® NorthernMax® Kit (Cat. no. AM1940).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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