# NorthernMax™ High Stringency Wash Buffer

Catalog Number AM8674

Pub. No. MAN0019799 Rev. A.0

**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 **thermofisher.com/support**.

## Product description

The Invitrogen™ NorthernMax™ High Stringency Wash Buffer (Cat. No. AM8674) is a proprietary formulation equivalent to 2X SSC or 2X SSPE wash buffers.

## Contents and storage

| Contents | Amount | Storage |
|---|---|---|
| NorthernMax™ High Stringency Wash Buffer[1] | 1 L | Room temperature |

[1] **Appearance**: Clear solution

## Wash Northern membranes

1. Add 20 mL per 100 $cm^2$ membrane of Low Stringency Wash Buffer (Cat. No. AM8673) at room temperature to the bag or tube.
2. Agitate for 5 minutes, remove and discard solution, then repeat.
   This serves to remove hybridization solution and free probe.
3. Preheat 40 mL of NorthernMax™ High Stringency Wash Buffer per 100 $cm^2$ membrane to the hybridization temperature indicated in the table following this procedure.
4. Use half of the wash buffer to wash the blot at the hybridization temperature for 15 minutes, with agitation.
5. Discard this solution, replace with the remaining half of the preheated wash buffer, and wash again for 15 minutes.
6. If a radiolabeled probe was used, remove the blots from the final wash and wrap them in plastic wrap or sheet protectors to prevent them from drying out.
   The blots may now be exposed to film for autoradiography.
   **Note:** Do not allow the blots to dry; you will be unable to strip the blot for subsequent use.

**IMPORTANT!** Discard radioactive material in accordance to federal, state, and local laws.

### Pre-hybridization and hybridization temperature by probe type

| Probe type | Prehybridation/ hybridation temperature |
|---|---|
| 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 42°C |

[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.
- **Physico-chemical testing**: pH, conductivity, density, refraction index

## 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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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