Dako
Stringent Wash (2X SSC)
for Nucleic Acid Blotting with Oligonucleotide Probes

Code S1803

Intended use
For Laboratory Use.

This product is for stringent washing of blots that have been hybridized with oligonucleotide probes. A 60 °C wash temperature is suggested to achieve stringent conditions for probes approximately 20 to 40 bases in length. The solution is optimized for use with the Dako Chemiluminescent System for Nucleic Acid Blotting (code K0626).

Presentation
Five packets containing powder to make 2X SSC, 0.1% SDS, Tris-HCl buffered stringent wash. Each packet makes 2 liters.

Precautions
1. For laboratory use.
2. Care should be taken to maintain stringent temperature throughout the procedure.
3. Do not let the blot dry out at any time after starting the procedure, as this may cause non-specific probe binding leading to high background.
4. Improper handling of blots may cause background spots and smudges. Handle the blot by the edges only, using clean forceps. Be careful not to scratch the membrane during handling.
5. Use prudent laboratory practices when handling reagents. This includes avoiding unnecessary contact, and using personal protective equipment such as chemical resistant gloves, eye protection, and lab coat.
6. Unused solution should be disposed of according to local, State and Federal regulations.
7. Safety Data Sheet available for professional users on request.

Storage
Store at Room Temperature.

Reagent preparation
Dissolve the entire contents of one packet in 1.9 L of distilled or deionized water while stirring uncovered in a glass beaker. If material does not go into solution after several minutes, gently heat the solution and continue to stir. Bring final volume to 2L with distilled or deionized water.

Unused solution may be stored at room temperature for 2–3 weeks. If precipitates form during storage, stir and/or heat gently to re-dissolve.

Procedure
1. Pre-warm a water bath to 60 °C.
2. Calculate the total volume of stringent wash required for the blot being washed. A total of 3 mL per cm² of membrane will be needed.
3. Heat the stringent wash to 60 °C in the heated water bath. Verify temperature of the solution with a thermometer.
4. Remove blot from hybridization solution and immerse in stringent wash solution. Wash the blot a total of 3 x 10 minutes, being careful to maintain the stringent temperature. Use 1 mL per cm² of membrane for each wash.

Blots can be washed in roller tubes or sealed bags in a hybridization oven or water bath to maintain temperature. Trays are not recommended, due to cooling of the wash solution caused by evaporation from the surface of the liquid. It is very important to maintain a sufficient stringent wash temperature in order to prevent non-specific cross hybridization of oligonucleotide probes. Because the effect of inadequate stringency varies between probes, the use of trays may be suitable for some but not all.

5. After washing, the blot is ready for detection. Blots hybridized with fluorescein-labelled probes may be detected using the Dako Chemiluminescent Detection System for Nucleic Acid Blotting (code K0626) (start with blocking step).

If signal is weaker than expected, stringency may be reduced by decreasing wash temperature. If non-specific cross hybridization is observed, stringency may be increased by elevating wash temperature.