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Product Information

10× Casein Blocking Buffer

Catalog Number **B6429**
Store at Room Temperature

Product Description

Membrane-based blotting applications that employ enzyme conjugates to generate a colorimetric or chemiluminescent signal require the use of an added blocking step to decrease the signal generated by non-specific binding. This 10x Blocking Buffer is a casein-based buffer designed to provide the highest possible signal-to-noise performance, while eliminating the inconvenience associated with the preparation of casein-based blocking buffers. The 10x Casein Blocking Buffer functions with both neutral and positively charged membranes and is recommended for both colorimetric and chemiluminescent detection.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store 10x Casein Blocking Buffer at room temperature. It is also stable when stored at 2–8 °C.

Casein may precipitate over time. This is normal, and should not impact the performance of the 10x Blocking Buffer (Catalog Number B6429). If precipitation is observed, resuspend the solids by inverting the bottle several times or by vortexing for a few seconds.

Reagents Required but Not Provided

(Catalog numbers have been given where appropriate)

Catalog Number	Product Name
T2663	1 M Trizma® HCl solution, pH 7.4
T6066	Trizma Base
T5941	Trizma HCl
S5150	5 M Sodium chloride solution
P9416	Polyoxyethylenesorbitan monolaurate (TWEEN® 20)
C0712	CDP-*Star*® Chemiluminescent Substrate Solution (for chemiluminescent detection)
B1911	BCIP®/NBT Liquid Substrate System (for colorimetric detection)

Preparation Instructions

1. 1 M Tris-HCl, pH 9.5: To 500 ml distilled deionized water, add 113.8 g Trizma Base (Product No. T6066) and 9.46 g Trizma HCl (Product No. T5941). Stir until dissolved. If necessary, adjust pH to 9.5 with concentrated HCl or NaOH. Adjust final volume to 1 L with distilled deionized water.

2. Wash Buffer 1 (0.1 M Tris-HCl pH 7.4, 0.15 M NaCl, 0.3% TWEEN 20): Prepare Wash Buffer 1 by combining 867 ml distilled deionized water, 100 ml of 1 M Tris-HCl pH 7.4 (Product No. T2663), 30 ml 5M NaCl (Product No. S5150), and 3 ml TWEEN 20 (Product No. P9416).

3. Wash Buffer 2 (0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl): Prepare Wash Buffer 2 by combining 880 ml distilled deionized water, 100 ml of 1 M Tris-HCl, pH 9.5 (see above) and 20 ml 5 M NaCl (Product No. S5150).

4. 1x Blocking Buffer: Prepare by diluting 10× Casein Blocking Buffer 10-fold with distilled deionized water.

Procedure

The following procedure details the processing of a previously hybridized membrane following the completion of stringency washes.

A. Blocking the Membrane

1. Wash the membrane with Wash Buffer 1 for five minutes with gentle agitation to remove any residual SDS carried over from the hybridization. Use 1–1.5 ml/cm^2 of membrane. Transfer the membrane to a clean container.

2. Incubate the membrane with 1x Blocking Buffer for 60 minutes at room temperature with gentle agitation. Use ~1 ml/cm^2 of membrane.

3. Proceed to Section B, Incubation with Antibody or Streptavidin Conjugate. Alternatively, the blocked membrane can be stored overnight covered in 1x Blocking Buffer at 2–8 °C.

B. Incubation of the Membrane with Antibody or Streptavidin Conjugate

1. Dilute the supplied antibody or streptavidin conjugate in 1X Blocking Buffer. The dilution to be used should be that recommended by the conjugate vendor (or empirically determined).

2. Incubate the membrane with diluted conjugate for 30 minutes at room temperature with gentle agitation. Use 1 ml/cm^2 of membrane.

C. Washing the Membrane

1. Using forceps, transfer the membrane to a clean container containing 1–1.5 ml Wash Buffer 1/cm^2 of membrane.

2. Wash the membrane for 5 minutes with gentle agitation.

3. Repeat steps 1 and 2 twice for a total of three washes with Wash Buffer 1.

4. Wash the membrane with Wash Buffer 2 for 5 minutes with gentle agitation. Use 1–1.5 ml/cm^2 of membrane.

5. Repeat step 4 for a total of two washes with Wash Buffer 2.

6. Continue to Section D1 for chemiluminescent detection or Section D2 for colorimetric detection.

D1. Detection of Labeled Probe with CDP-Star Chemiluminescent Substrate Solution

1. Using forceps, transfer the membrane to a clean, appropriately sized container.

2. Using aseptic technique, add CDP-*Star* to the membrane (50 µl/cm^2 membrane).

3. Incubate for 5 minutes at room temperature with gentle agitation to allow CDP-*Star* to fully cover the membrane.

4. Using forceps, remove the membrane and drain off any excess CDP-*Star* onto an absorbent material. Do not let the membrane dry out.

5. Transfer the membrane to a development folder, with the sample side up. Place into a light-tight film cassette.

6. Expose membrane to film from 30 seconds to overnight at room temperature. Exposure time should be adjusted accordingly to achieve the highest signal-to-noise ratio.

7. Develop film as per manufacturer's instructions.

D2. Colorimetric Detection of Labeled Probe with BCIP/NBT Liquid Substrate System

1. Using forceps, transfer the membrane to a clean, appropriately sized container.

2. Using aseptic technique, add BCIP/NBT to the membrane (100–1,000 µl/cm^2 membrane).

3. Develop for 5–60 minutes at room temperature with gentle agitation.

4. Wash the membrane with distilled deionized water for 5–15 minutes to quench development.

5. Dry membrane at 60–80 °C for at least 5 minutes.

6. Store membrane protected from direct light.

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TWEEN is a registered trademark of Croda International PLC

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