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Product Information

Casein Blocking Buffer
for Northern and Southern blotting
powder blend

Catalog Number **C7594**
Store at Room Temperature

Product Description

In order to specifically detect an antigen immobilized on a solid support, the unoccupied sites on the support must be blocked. Blocking of the non-specific sites may be accomplished by a variety of protein and detergent solutions. However, the blocking solution must be compatible with the detection system.

This Casein Blocking Buffer is compatible with a variety of detection systems, including fluorescein and DIG detection systems. Several studies cite use of this product.[1-5]

Reagents Required But Not Provided

(Catalog Numbers given where appropriate)

- Phosphate Buffered Saline (PBS) with 0.05% (v/v) TWEEN® 20 (PBS-T), e.g., Catalog Number P3563
- Protein probe or antibody
- 0.1 M Trizma®, 0.1 M NaCl, pH 9.5 (for Southern blotting)

Preparation Instructions

1. Dissolve the contents of one container of the Casein Blocking Buffer in 800 mL of deionized water.
2. Once the contents are dissolved, add deionized water to 1,000 mL and stir to mix.

Storage/Stability

Store the powder at room temperature. After reconstitution, store the Casein Blocking Buffer at 2–8 °C to avoid bacterial contamination. Solutions may be kept up to one week at 2–8 °C following reconstitution.

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.

Procedures

Recommended procedure for Southern blot[6] blocking, probing, and detection:

1. Transfer and crosslink labeled nucleic acid to a nitrocellulose, nylon, or positively charged nylon membrane.
2. Incubate the membrane with Casein Blocking Buffer for 60 minutes (0.6 mL/cm^2) at ambient temperature, or for 30 minutes at 37 °C with gentle agitation. Also, blocking can be accomplished overnight at 2–8 °C.
   (Note: Casein Blocking Buffer is not suitable for blocking PVDF membranes.)
3. The membrane may now be probed with either protein conjugates or antibodies specific to the label of interest. The protein conjugate or antibody should be diluted with PBS-T. An initial concentration of 1-10 ng/mL is suggested when using streptavidin-alkaline phosphatase (Catalog Number S2890) or streptavidin-peroxidase (Catalog Number S5512) as the protein conjugate.
4. Incubate the membrane with protein conjugate or antibody solution (0.6 mL/cm^2) for 30-60 minutes at ambient temperature with gentle agitation.
5. Wash the membrane, with gentle agitation, 3-5 times for five minutes each with PBS-T.
6. If the protein conjugate uses alkaline phosphatase (AP) as the enzyme, it will be necessary to follow the PBS-T washes with three washes for 3 minutes each with 0.1 M Trizma, 0.1 M NaCl, pH 9.5. The Trizma washes remove any residual phosphate and equilibrate the membrane to an alkaline pH for assaying AP.
   If the protein conjugate uses horseradish peroxidase (HRP) as the enzyme, the membrane is ready to be exposed to substrate after the PBS-T washes.
7. The membrane may now be exposed to chromogenic or chemiluminescent substrate as per the manufacturer's instructions.

Recommended procedure for Western blot[7-9] blocking, probing, and detection:

1. Transfer and crosslink labeled nucleic acid to a nitrocellulose membrane.
2. Incubate the membrane with casein blocking buffer for 10 minutes (0.6 mL/cm^2) at ambient temperature or for 30 minutes at 37 °C with gentle agitation. Blocking can be accomplished overnight at 2–8 °C.
3. Dilute the primary antibody in Casein Blocking Buffer. A common dilution for primary antibodies is 1:1,000, but may be varied as needed. Dilutions may vary from 1:100 to 1:100,000, or higher. The researcher must determine the optimal dilution factor.
4. Incubate the membrane with the primary antibody (0.6 mL/cm^2) for 1-16 hours at 2–8 °C with gentle agitation.
5. Wash the membrane, with gentle agitation, 3-5 times for five minutes each with PBS-T.
6. Dilute the enzyme-antibody conjugate in Casein Blocking Buffer. Incubate the membrane for 30-120 minutes. After the incubation, wash the membrane with gentle agitation, 5-6 times for five minutes each with PBS-T.
7. The membrane may now be exposed to chromogenic or chemiluminescent substrate as per the manufacturer's instructions.

Suggestions for colorimetric detection of labeled nucleic acid:

1. The membrane should be exposed to the colorimetric substrate until a positive signal is seen. As background begins to develop, the reaction should be stopped. The membrane should be exposed to the substrate for no longer than 60 minutes.
2. For colorimetric HRP substrates, the reaction may be stopped by removal of the substrate and transfer of the membrane to a solution of 0.1% sodium azide with 1% SDS in either PBS or TBS (Tris-buffered saline).
3. For AP substrates, the reaction may be stopped by removal of the substrate and transfer of the membrane to a solution of 0.3 M sodium phosphate, pH 5.5.

Suggestions for chemiluminescent detection of labeled nucleic acid:

1. Following exposure to the substrate, the excess substrate should be blotted off, and the membrane transferred to a solid support. Transfer of the membrane to a "page protector", slightly larger than the membrane itself, is recommended.
2. The supported membrane should then be placed within a heat-sealable bag. Using gentle pressure, smooth out air bubbles between the membrane and the plastic bag by rolling a glass test tube or pipette over the contained membrane. Seal the bag and wipe off any excess substrate from the outside of the bag.
3. Place the contained membrane into a film cassette.
4. Expose the contained membrane to film.
5. Initially, an exposure of 1 minute should be used. However, if no signal is seen, expose the membrane to film for longer times. If excess signal is seen, use as short an exposure as technically possible. See the Troubleshooting Guide for other hints.

Suggestions for Western blot detection of labeled nucleic acid:

1. Dilutions of the enzyme-antibody conjugate depend on the substrate used for subsequent detection and should be optimized by the researcher. General guidelines for dilutions are 1:5,000 for chromogenic substrates and 1:50,000 for chemiluminescent substrates. These dilution ratios are based on an initial concentration of ~1 mg/mL enzyme-antibody conjugate.
2. In sensitive systems, fingerprints will show up. Use powder-free gloves at all times. Avoid the use of forceps with ridges, as these also tend to show up.
3. If using a biotin-avidin system for detection, the blocking reagents should be free of biotin. Casein Blocking Buffer is not recommended for use in these systems, since milk contains large and variable amounts of biotin.
4. Casein Blocking Buffer is not recommended for use in systems detecting phosphoproteins, because of the presence of phosphorylated proteins. Gelatin blocking buffer (e.g. Catalog Number G7663) is recommended for those systems.
5. To verify the quality of the secondary antibody, perform a "blank" membrane, in which the primary antibody is omitted. If background is present without the primary antibody, either use a different blocking reagent, or dilute the conjugate further.
6. The antibodies that are used to detect antigens can usually be removed by immersing the membrane in a buffer containing 100 mM glycine, pH 2.3 for 30 minutes with agitation. This is not a universally applicable procedure, as some antigens may dissociate from the membrane under such conditions, so subsequent probing will be faint or non-existent. Many researchers prefer to prepare membranes in parallel if possible, thereby avoiding the uncertainty of this "stripping" step.

References

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8. Dunbar, B.S., (ed.) Protein Blotting: A Practical Approach, IRL Press, NY, p. 67-70 (1994).
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Troubleshooting Guide

Reagent	Possible Cause	Remedy
No Signal	No target present	If the labeled nucleic acid is not present on the membrane, it cannot be detected. Include a positive control with the next assay.
	No target protein present	Verify transfer by visualizing proteins on the membrane using a Ponceau S solution (Catalog Number P7170). If possible, a positive control should always be run to insure that the components are functioning.
	Overblocking	Masking of a signal can occur if the blocking reagent is used at too high a concentration. A dilution of 1:1 to 1:3 may be done to decrease the concentration. If the problem persists, different blocking reagents should be tried.
	Interference from contaminants in blocking reagents (e.g., biotin systems)	If the blocking reagent is contaminated with biotin, the free biotin will bind the streptavidin conjugate, so that it will not be available for detection.
	Interference from contaminants in blocking reagents (e.g., phosphoprotein systems)	If the blocking reagent contains phosphoproteins, the phosphate sites will bind the alkaline phosphatase conjugate, so that it will not be available for detection.
	Inadequate exposure time using chemiluminescence system	The first exposure should be 1 minute. If no signal is seen, expose for longer times, e.g. 5 minutes, 10 minutes. If excess signal is seen, try as short an exposure as practical (down to 1 second) without using a cassette.
	Enzyme conjugate may have lost enzymatic activity	Determine if the enzyme conjugate is active.
High background	Too much antibody or protein conjugate	Perform a titer of the antibody or protein conjugate until an acceptable signal to noise ratio is obtained.
	Inappropriate blocking reagent	Increase the concentration of the blocking reagent by preparing the reagent with one-half the recommended volume of water. In addition, some antibodies may crossreact with certain blocking reagents. To test for this possibility, prepare a "blank" membrane that does not contain the primary antibody.
	Inappropriate blocking protocol	Increase the blocking time and increase the blocking temperature to 37 °C.

	Inappropriate wash protocol	Increase the number of washes.
	Overincubation in colorimetric substrate solution	Decrease the staining time. The membrane should be exposed to the colorimetric substrate until a positive signal is seen. However, as the background begins to develop, the reaction should be stopped. For colorimetric substrate: Incubate for 5-10 minutes or whenever bands are visible. The time required may be increased or decreased, but should not exceed 60 minutes. To stop reactions: • For alkaline phosphatase substrates, use a solution of 0.3 M sodium phosphate, pH 5.5. • For HRP substrates, wash the membrane with 0.1% sodium azide with 1% SDS in either TBS (Tris-buffered saline) or PBS (phosphate-buffered saline).
	Inappropriate film	Switch to film designated for chemiluminescent detection, such as Kodak® Biomax® Light, MS, and MR.
	Aggregated protein or antibody conjugate	Filter the conjugate through a 0.2 micron cellulose acetate filter, or centrifuge the conjugate solution at 10,000 × *g* for 10 minutes and use the supernatant.
Extraneous spots	Too much antibody or protein conjugate	Perform a titer of the antibody or protein conjugate until an acceptable signal to noise ratio is obtained.