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Product Information

Gelatin Blocking Buffer
for Western blotting, powder blend

Catalog Number **G7663**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

In order to specifically detect an antigen or target molecule immobilized on a solid support, unoccupied binding sites on the support must be blocked against binding by probe and detection molecules. Otherwise, nucleic acid probes, antibodies, and detection enzymes will bind randomly on the membrane as well as to the target molecules. Blocking these sites is one way to minimize background noise.

Blocking of non-specific protein binding sites may be accomplished by a variety of protein and detergent solutions. However, the blocking solution must be compatible with the detection system. This Gelatin Blocking Buffer is compatible with a variety of detection systems, including biotin, fluorescein, and DIG detection systems. Target molecules can be immobilized on nitrocellulose, nylon, and charge-modified nylon membranes. Gelatin blocking buffer is **not** suitable for blocking PVDF membranes.

This product has been used for binding assays in 96 well plates.[1]

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® with 0.1 M NaCl, pH 9.5 (for Southern blotting)

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.

Preparation Instructions

1. Dissolve the contents of one container of the Gelatin 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 Gelatin Blocking Buffer at 2–8 °C to avoid bacterial contamination. Solutions may be kept up to one week at 2–8 °C following reconstitution.

Procedures

Note: In sensitive systems, fingerprints will show up on the membrane. Use powder-free gloves at all times. Avoid the use of forceps with ridges, as contact points also tend to show up.

Recommended procedure for Southern blot[2] 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 Gelatin Blocking Buffer (0.6 mL/cm^2) for 60 minutes at ambient temperature, or for 30 minutes at 37 °C with gentle agitation. Blocking can also be accomplished overnight at 2–8 °C.
   (Note: If a labeled nucleic acid probe is to be used to detect an unlabeled nucleic acid target, the membrane must first be blocked with a suitable single-stranded DNA, according to a preferred procedure.)
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 with 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[3-5] blocking, probing, and detection:

1. Transfer and crosslink labeled nucleic acid to a nitrocellulose membrane.
2. Incubate the membrane with Gelatin 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 Gelatin 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 Gelatin 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 substrate for no longer than 60 minutes.
2. For colorimetric HRP substrates, the reaction may be stopped by removal of 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 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 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 immobilized protein:

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 1:5000 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. 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.
3. 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

1. Lu, Z. et al., J. Biol. Chem., **289(50)**, 34530-34542 (2014).
2. Sambrook, J. *et al*., *Molecular Cloning: A Laboratory Manual, Second Edition*. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY), pp. 9.47-9.50 (1989).
3. Bjerrum, O.J., and Heegaard. N.H.H., *CRC Handbook of Immunoblotting of Proteins*: *Volume I, Technical Descriptions*. CRC Press (Boca Raton, FL), pp. 229-236 (1988).
4. Dunbar, B.S. (ed.), *Protein Blotting: A Practical Approach*. IRL Press (New York), pp. 67-70 (1994).
5. Fortin, A. *et al*., *Biochem. Cell Biol*., **72(5-6)**, 239-243 (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.
	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 cross-react 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.
Extraneous spots	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.