KPL APPLICATION NOTE

A Comparison of Blocking Solutions Used in Western Blotting

By Amy L. Connolly and Helen M. Mahy
KPL Research and Development

The sensitive detection of proteins of interest in Western blotting and immunoassay applications is dependent on a number of elements, from the specificity of the primary antibody to the reduction of competing biomolecules present in the assay. To minimize non-specific binding, blocking solutions are a critical component of all detection protocols.

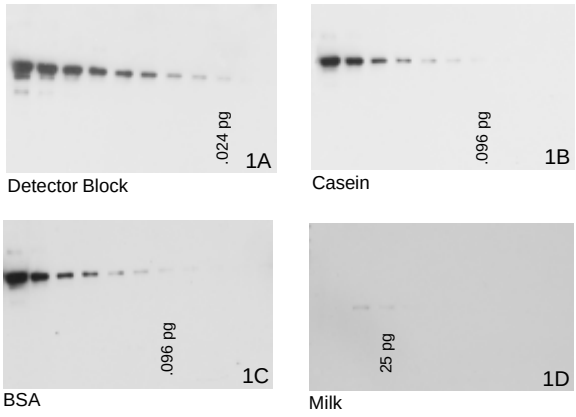
A variety of products are readily available for this purpose, the most common being non-fat dry milk, bovine serum albumin (BSA) and casein. However, researchers are frequently met with concerns when using these solutions. The issues include signal quenching, inconsistent results arising from lot-to-lot variability, and incompatibility with certain assay formats (i.e., membrane, enzyme, primary antibody). For these reasons, KPL has developed Detector™ Block (Cat. No. 71-83-00), a unique formulation that delivers a comparatively higher signal:noise ratio than traditional blocks across applications, including Southern, Northern, and Western blotting. In this Application Note, the differences in performance between blocking solutions are compared on Western blots.

Western Blot Analysis

Side-by-side studies were conducted to demonstrate relative sensitivity achieved when using the various blocks in Western blotting. Mouse IgG was electrophoresed on a nonreducing Tris-Glycine gel and transferred to membrane. Detector Block was developed and optimized for use with PVDF, nitrocellulose, and nylon but for the replicate experiments shown here, KODAK BIOMAX Multi-Blot Kit for Proteins was used. [NOTE: Sensitivity will vary depending on the antigen-antibody system detected.] Blots were detected using KPL's Protein Detector LumiGLO® Western Blotting Kit and HRP-streptavidin, substituting in various block solutions. The detection protocol is as follows:

1. Blots were blocked overnight at 4°C in respective Blocking Solutions prepared as instructed by manufacturer [1X Detector Block, 1X Casein, BSA, Milk].
2. Blots were equilibrated to room temperature for approximately 15 minutes.
3. Biotinylated Goat anti-Mouse antibody was diluted 1:1000 directly into the block solution. Blots were incubated for 1 hour at room temperature on a rotator.
4. Blots were washed 3 times at 5 minutes each with 1X Wash Solution.
5. HRP-Streptavidin was diluted 1:1000 in each of the fresh block solutions and added to the appropriate blot. Blots were incubated for 1 hour at room temperature on a rotator.
6. Blots were washed 3 times at 5 minutes each with 1X Wash Solution.
7. LumiGLO was prepared and added to each blot for 1 minute. Excess solution was blotted off with filter paper. Blots were placed in a sheet protector and exposed to KODAK BIOMAX Light Film for 10 minutes.

Figure 1.



Sensitivity of protein detection using different blocking solutions (Panel 1A: Detector Block; 1B: Casein; 1C: BSA; 1D: Milk). 2-fold serial dilutions of mouse IgG were separated, transferred, and subsequently detected with LumiGLO. The sensitivity achieved with Detector Block, Casein and BSA were 24 fg, 96 fg, and 96 fg, respectively. Similar detection limits were not feasible with Milk, capable of detecting only 25 pg of protein.

As illustrated in Figure 1, sensitivity of blots blocked with Detector Block surpassed that of competitor products, detecting protein as low as 24 femtograms. Other blocks such as the traditional milk tend to reduce signal by virtue of the abundance of multiple proteins inherent in the solution. Because milk may contain varying amounts of biotin, this block should be avoided in biotin-streptavidin systems. Biotin present in milk competes with the biotinylated primary for the streptavidin conjugate reducing sensitivity.

To resolve the issues with milk, casein, a purified milk protein, has emerged as a frequently used alternative in the blocking of membranes. However, blots blocked with casein remain less sensitive than those using Detector Block. In addition, casein may not be an adequate universal blocking agent, as it does not perform well on all types of membranes, specifically nylon, with both HRP and AP enzyme conjugates. Detector Block has been developed to provide the same high quality results in Southern, Northern and Western blots regardless of the membrane or enzyme used for detection.

Likewise, immunoassays employing Detector Block require less optimization of the primary antibody concentration, yet still deliver clean results. This is not the case for all blocks, and is of specific concern when utilizing BSA in the detection of certain antigens. The concentration of primary antibody and blocking time must be adjusted by system to minimize the appearance of background. Figure 2 shows the detection of human matrix metalloproteinase 2 (MMP-2) using the Protein Detector LumiGLO Western Blotting Kit and a rabbit anti-MMP-2 primary antibody, comparing the performance of Detector Block vs. BSA. In this system, BSA is not compatible with the primary antibody at the manufacturer's recommended use dilution whereas optimization of the antibody is not required when using Detector Block.

Figure 2.

Detector Block

BSA

Comparison of Detector Block and BSA in systems detecting Human matrix metalloproteinase 2 (aka gelatinase A, 72 kD type IV Collagenase A). MMP-2 was detected using 20 ng/ml of rabbit anti-MMP-2 primary antibody and the Protein Detector LumiGLO Western Blotting Kit with HRP anti-rabbit conjugate, 10 minute film exposure.

Conclusions

Researchers are accustomed to evaluating different blocking solutions for each immunoassay in order to select the one that provides the strongest signal with minimum background. Detector Block reliably facilitates the sensitive detection of specific membrane-bound protein and nucleic acids. Its novel formulation overcomes the many issues associated with the once-standard blocks. As a support reagent or a critical component of KPL's Detector Non-Radioactive Blotting Kits, Detector Block is an integral part of any immunoassay.

Related Products:

Protein Detector™ LumiGLO Western Blot Kit
Catalog No. 54-12-50

Protein Detector™ BCIP/NBT Western Blot Kit
Catalog No. 55-11-50

Protein Detector™ TMB Western Blot Kit
Catalog No. 54-11-50

ExpressDetector™ Ni-HRP Chemiluminescent Blotting Kit
Catalog No. 54-31-02

For technical support or order information, contact KPL at 800-638-3167, 301-948-7755 or visit us at www.kpl.com.

KPL, Inc. Gaithersburg, MD 20878 USA
800-638-3167 301-948-7755
FAX 301-948-0169 www.kpl.com