Datasheet for ABIN925618

Blocking Buffer for Fluorescent Western Blotting

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Overview

Quantity:	500 mL
Application:	Blocking Reagent (BR), Labeling (Lbl), Western Blotting (WB)

Product Details

Purpose:	Blocking Buffer for Fluorescent Western Blotting
Specificity:	Blocking buffer was prepared using ultra pure reagents dissolved in pharmaceutical grade water (WFI) and consists of a proprietary protein formulation in TRIS buffered saline at pH 7.6 with thimerosal added as an antimicrobial agent.
Characteristics:	Western Blot Blocking Solution is specifically designed for western blotting using fluorochrome conjugated antibodies. Pure nitrocellulose membrane is recommended for maximum performance. Other membranes, such as PVDF or nitrocellulose embedded in a support can be used, but may generate elevated backgrounds. Protein should be transferred from gel to membrane using standard protocols. Blocking buffer can be used for membrane blocking and to dilute both primary and secondary antibodies. Western Blot blocking buffer is suitable for use with fluorescent western blot imaging systems produced by Bio-Rad Laboratories, GE Healthcare, Alpha Innotech, FujiFilm Life Science, Licor Biosciences, UVP and Syngene.
Purification:	This product was aseptically filtered through a Millipore 0.22 micron filter into clean, pre-sterilized containers. The product was tested on trypticase soy agar for 24 hours, 48 hours and 72 hours and was found to be negative for bacteria.
Sterility:	Sterile filtered

Application Details

Application Notes:	Western Blot : User Defined Other Dilution: Optimum performance is achieved using this product undiluted. However, this blocking solution can often be diluted 1:1 in TBS without a significant loss of performance.
Comment:	Synonyms: Multiplex Blocking Buffer, Fluorescent Blocking Buffer, Blocking Solution, Blocking Buffer Western Blot, IRDye Western Blot Blocking Buffer, Alexa Dye Blocking Buffer, DyLight Blocking Buffer
Protocol:	Fluorescence technology is widely used to detect proteins in both the visible and near-infrared ranges. This product allows for superior signal detection and lower background noise when fluorochrome conjugated antibodies are used to visualize proteins in western blotting and other applications. Antibody conjugates prepared with IRDye® 800 and IRDye® 700DX (Licor), Cy2™, Cy3™, Cy3.5™, Cy5™ and Cy5.5™ (GE Healthcare), DyLight™405, DyLight™ 549, DyLight™ 649, DyLight™ 680, and DyLight™ 800 (Thermo Fisher/Pierce) and Alexa Fluor® 488, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 647 and Alexa Fluor® 680 (Invitrogen/Molecular Probes) have been validated on various platforms using this product with superior results compared to other commercially available products. In the infrared range, where little to no autofluorescence occurs, specific signal is sharply evident from any background giving the best possible signal-to-noise ratio. This allows for detection levels in the picogram range which rivals the sensitivity of chemiluminescence on film for western blotting. Superior results are also seen when this product is used for simultaneous labeling (multiplex) in western blots or microscopy using various fluorochrome combinations for multicolor imaging. Membranes blocked with this product can be dried and are very stable. Membranes that are stored protected from light can be re-washed and/or rescanned.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	1 X
Preservative:	Thimerosal (Merthiolate)
Precaution of Use:	This product contains thimerosal (merthiolate): a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C
Storage Comment:	Store Blocking Buffer at 4° C prior to opening. DO NOT FREEZE.

Publications

Product cited in:

Spence, Dube, Uezu, Locke, Soderblom, Soderling: "In vivo proximity proteomics of nascent synapses reveals a novel regulator of cytoskeleton-mediated synaptic maturation." in: **Nature communications**, Vol. 10, Issue 1, pp. 386, (2019) (PubMed).

Führer, Heine, Helmsing, Goy, Heidepriem, Loeffler, Dübel, Gerhard, Hust: "Development of Neutralizing and Non-neutralizing Antibodies Targeting Known and Novel Epitopes of TcdB of Clostridioides difficile." in: **Frontiers in microbiology**, Vol. 9, pp. 2908, (2018) (PubMed).

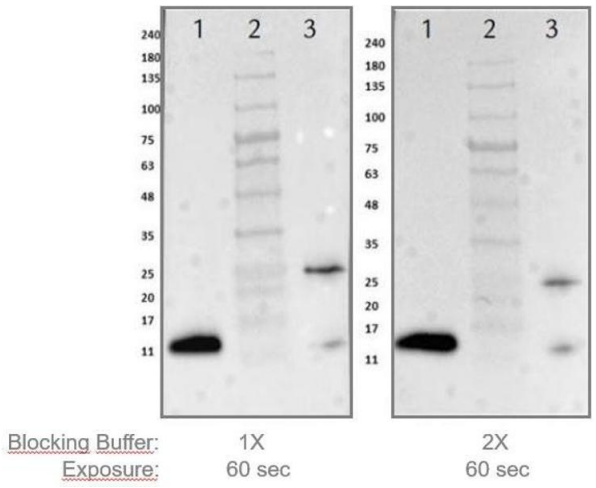
Smith, Saul, Barton, Luna: "Generation and characterization of monoclonal antibodies that recognize human and murine supervillin protein isoforms." in: **PLoS ONE**, Vol. 13, Issue 10, pp. e0205910, (2018) (PubMed).

Pereira, Bacman, Arguello, Zekonyte, Williams, Edgell, Moraes: "mitoTev-TALE: a monomeric DNA editing enzyme to reduce mutant mitochondrial DNA levels." in: **EMBO molecular medicine**, Vol. 10, Issue 9, (2018) (PubMed).

Yang, Mott, Sutiwisesak, Lu, Raso, Stowell, Babunovic, Lee, Carpenter, Way, Fortune, Behar: " Mycobacterium tuberculosis-specific CD4+ and CD8+ T cells differ in their capacity to recognize infected macrophages." in: **PLoS pathogens**, Vol. 14, Issue 5, pp. e1007060, (2018) (PubMed).

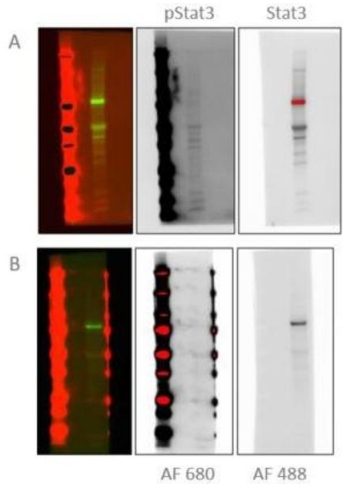
There are more publications referencing this product on: Product page

Validation report #104437 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)



Western Blotting

Image 1. Western Blot Comparison of 1X and 2X Universal Blocking Buffer. Lane 1: reduced human NAG1 protein 0.1µg. Lane 2: Prestained Molecular Weight Marker 5µL. Lane 3: non-reduced human NAG1 protein 0.1µg. Blocking Buffer: Left blot 1X (ABIN925618), Right blot 2X (ABIN6953293) for 30 min at RT. Primary Antibody: rabbit anti-NAG1 biotin conjugated antibody (ABIN1043807) at 1µg/mL overnight at 2-8°C. Secondary Antibody: goat anti-rabbit antibody HRP conjugated (ABIN102010) at 1:70,000



for 30 min at RT. Expect: ~14kDa for NAG1.

Western Blotting

Image 2. Comparison of blocking buffer on multiplex fluorescent detection of pStat3 and Stat3. Alternating samples of the Precision Plus Protein Unstained Standards and 20 µg of brain lysate were subjected to SDS-PAGE, transferred to LF-PVDF, and cut into separate membranes for use of different blocking buffers. The membranes were blocked in Blocking Buffer for Fluorescent Western Blotting (A) or LI-COR Odysses blocking buffer (B). After a 30 min incubation in blocking buffer, the membranes were incubated with rabbit anti-pStat3 and mouse anti-Stat3 antibodies diluted in 5% BSA in PBS/T, and incubated overnight at 4°C with agitation. Approximately 18hr later, the primary antibody solutions were discarded, the membranes were washed 3x for 5min in PBS/T, and the primary antibodies were detected with Alexa Fluor 680 (pStat, 1:4,000 dilution) or Alexa Fluor 488 (Stat3, 1:4,000 dilution) in the same diluent as the primary antibody.