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Datasheet for B501-0500

Blotto Immunoanalytical Grade (Non-Fat Dry Milk)

Overview

Description:	Blotto Immunoanalytical Grade (Non-Fat Dry Milk) - B501-0500
Item No.:	B501-0500
Size:	500 g
Applications:	WB, Biochemical Assay, ELISA, IP

Product Details

Background:	Rockland Immunochemicals produces the following blocking buffer reagents: BSA, BLOTTO, BLOTTO A, BLOTTO B and Blocking Buffer for Fluorescent Western Blotting (see related products below). These products are intended to block non-specific binding of proteins in various immunological assays. Typically, western blotting and ELISA methods call for the addition of a blocking agent prior to the addition of primary antibody.
Synonyms:	Blotto Immunoanalytical Grade Non-Fat Dry Milk, Blocking reagent for western, antibody dilution buffer

Target Details

Purity/Specificity:	This product contains highly purified immunoanalytical grade non-fat dry milk.
Relevant Links:	• B501-0500 SDS

Application Details

Tested Applications:	WB
Suggested Applications:	Biochemical Assay, ELISA, IP (Based on references)

Application Note:	Use Rockland's BLOTTO whenever procedures call for immunoanalytical grade non-fat dry milk. Alternative blocking agents such as BLOTTO A (solution of TBS, 5% non-fat dry milk and 0.05% Tween-20) and BLOTTO B (solution of TBS, 1% non-fat dry milk, 1% BSA and 0.05% Tween-20) are also available. BLOTTO A is a convenient general purpose blocking agent. BLOTTO B is recommended for blocking when phospho-specific antibodies are used. BLOTTO A and BLOTTO B provide Tween-20 in a separate vial in liquid form (p/n TW0025). Users should consider the addition of protease inhibitors and phosphatase inhibitors when required. Typically 50 mM NaF can be added to BLOTTO solutions to inhibit phosphatase activity.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	User Optimized
IF:	User Optimized
WB:	User Optimized

Formulation

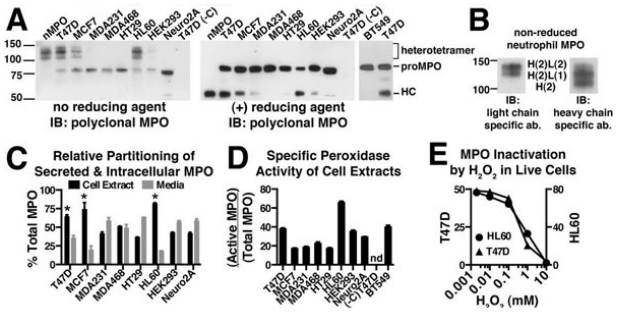
Physical State:	Lyophilized
Buffer:	None
Preservative:	None
Stabilizer:	None
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

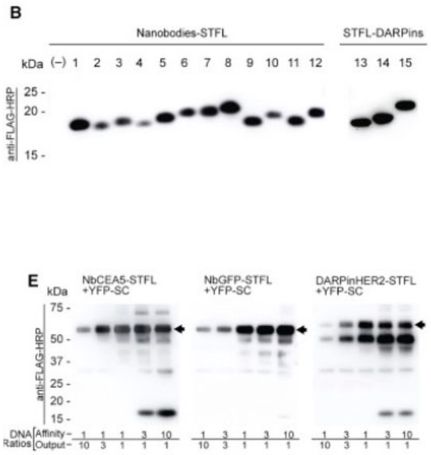
Shipping Condition:	Ambient
Storage Condition:	Store container at room temperature prior to opening. After reconstitution, use blocking buffers immediately. Dilute solutions may be stored at 4° C for up to four (4) days. Solutions containing BLOTTO may be frozen.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

Western Blot
Screening multiple cell lines reveals that T47D and MCF7 cells efficiently process recombinant MPO into the mature heterotetrameric form. (A) Cell extracts were made from the indicated stable cell lines and the total MPO concentration in each extract quantitated by ELISA. Cell extract containing



2 ng of total MPO was run on Bis-Tris gels in the absence or presence of reducing agent and immunoblotted with a multi-epitope polyclonal MPO antibody to assess the MPO species present in each cell line. Under non-reducing conditions the MPO heterotetramer migrates as three bands between the 100–150 kDa markers. Under reducing conditions, the heterotetramer falls apart and we instead observe the heavy chain (HC) of MPO that migrates just above the 50 kDa marker. Monomeric proMPO migrates just above the 75 kDa marker and shifts slightly upward upon reduction of the intra-molecular disulfide bonds. Untransfected T47D cells (-C) were included as a negative control. (B) The three bands between the 100–150 kDa markers that represent fully processed MPO on non-reducing SDS PAGE were further analyzed to determine if subunit structure was responsible for their distinct gel mobilities. Purified nMPO was run on non-reducing SDS-PAGE and duplicate blots were probed with antibodies specific for either the light chain (L) or heavy chain (H) of MPO. The subunit stoichiometry consistent with the relative immunoreactivity of each band is indicated between the two panels. (C) MPO concentrations in 24 h conditioned media and corresponding whole cell extracts were determined by ELISA and the relative amounts of secreted versus cellular MPO were calculated for each cell line. (D) Whole cell extracts from each cell line were assayed for peroxidase activity and total MPO content and the relative specific activity (Active MPO/Total MPO). (E) Comparison of the sensitivity of native (HL60) and recombinant (T47D) MPO to inactivation by H2O2. Live cells were treated with increasing concentrations of H2O2 in the growth media for 1.5 h at which time further inactivation was stopped by the addition of catalase. Specific peroxidase activity was measured in cell extracts generated from treated cells as in panel D. IC50 values are 2.8 mM (HL60) and 0.96 mM (T47D-MPO). (C, D & E) Assay points are triplicate measurements and plotted as mean ± SE. The data shown is representative of at least three independent experiments. Membranes were blocked with 4% non-fat dry milk and plates were blocked 2% non-fat dry milk (p/n B501-0500). (nd) None detected. Fig 2. PMID: 26890638.



Western Blot
FD-CF Production of On-Demand Affinity Products
(B) Anti-FLAG WB showing FD-CF expression of 12 Nanobodies and three DARPins containing ST-FLAG tags (STFL). Specific antigen targets are: (1) CEA5, (2) dengue Virus NS1, (3) GFP, (4) HIV p23-gag, (5) norovirus capsid VP1, (6) rotavirus capsid VP6, (7) C. difficile exotoxin TcdA, (8) P. falciparum VAR2CSA, (9) GLUT1, (10) mCherry, (11) Vimentin, (12) Glycophorin A, (13) HER-2, (14) VEGF-A, and (15) epCAM. (–) indicates a DNA null control reaction.
(E) Anti-FLAG WB showing one-pot manufacturing of affinity-output proteins, produced by mixing DNA templates encoding different affinity components (left, anti-CEA5-STFL Nanobody; middle, anti-GFP-STFL Nanobody; right, STFL-anti-HER2 DARPin) with DNA encoding the YFP-SC output component in a single FD-CF reaction. Template ratios are shown below. Membrane was blocked in 4% Blotto (p/n B501-0500) + 2% cold water fish gelatin. Figure 4. PMID: 27662092.

Bottle
Blotto Immunoanalytical Grade (Non-Fat Dry Milk)

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