AdvanBlock™-Chemi Blocking Solution

Blocking solution and antibody-antigen signal enhancer all-in-one for chemiluminescent Western blots

AdvanBlock-Chemi is a novel blocking solution, optimized to enhance specific antibody-antigen interactions for chemiluminescent Western Blots. This all-in-one blocking and antibody incubation solution is designed to improve sensitivity and decrease overall background. Non-specific binding caused by low quality antibodies is reduced while signal from the specific antibody-antigen complex is stabilized and enhanced. Provided as a convenient ready-to-use solution intended to directly replace other commonly used blocking buffers for Western Blotting.

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Maximum sensitivity

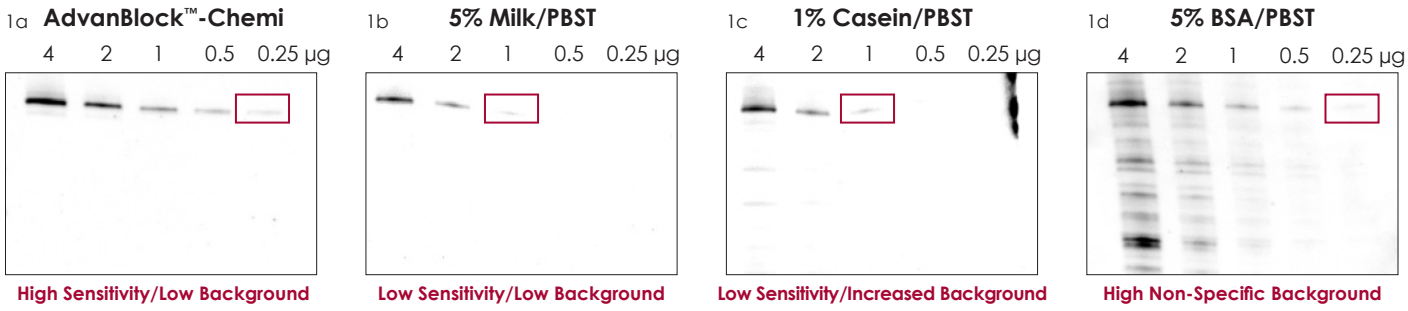

Figure 1. Sensitivity and specificity of phospho-protein detection with various blocking buffers. 2-fold serial dilutions of IFNα-treated HeLa lysate were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The blots were blocked with various blocking buffers before incubation with anti-phospho STAT-3 (Cell Signaling Technology #9145S). Signal was detected with WesternBright® ECL. AdvanBlock-Chemi yields maximum sensitivity without increased non-specific binding.

Effect of blocking buffer on sensitivity

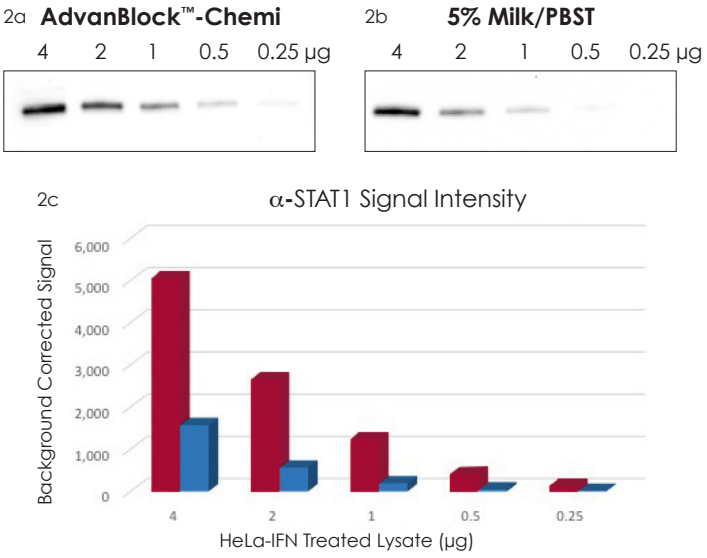

Figure 2. Effect of blocking buffer on sensitivity. 2-fold serial dilutions of IFNα treated HeLa lysate were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The blots were blocked with AdvanBlock-Chemi and milk-PBST blocking buffers before incubation with anti-STAT-1 (Millipore #06-501). Signal was detected with WesternBright ECL. The sensitivity of detection with AdvanBlock-Chemi was much greater than with Milk-PBST.

Advantages

- ENHANCES CHEMILUMINESCENT SIGNAL
- DECREASES BACKGROUND
- DECREASES NON-SPECIFIC BINDING
- READY-TO-USE SOLUTION

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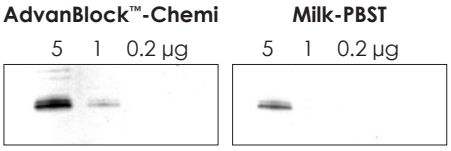
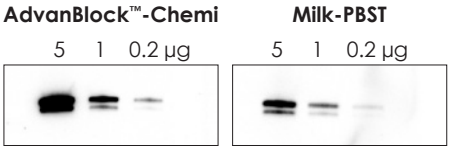
AdvanBlock-Chemi compared to 5% Milk-PBST

Rabbit α ERK-2: GenScript #A01194



Rabbit α pSTAT-1: CST #7649S



Mouse α Tubulin: Millipore #MAB5564

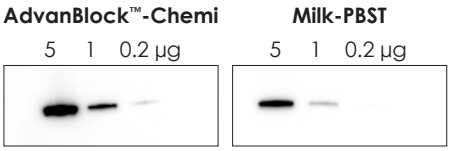


Figure 3. Enhanced sensitivity for modified, low and high abundance proteins. 5-fold serial dilutions of IFNα-treated HeLa lysate were separated by SDS-PAGE and transferred to a membrane. The blots were blocked with AdvanBlock-Chemi or 5% Milk-PBST before incubation with various antibodies diluted in corresponding blocking buffer. Signal was detected with WesternBright ECL. AdvanBlock-Chemi consistently yields improved sensitivity without optimization.

Antibody	Manufacturer	Catalog Number	Membrane Material	Sample Type	Fold Increase (Signal-Background)
STAT-1	Millipore	06-501	PVDF	HeLa	4.1
pSTAT-1	CST	7649S	PVDF	HeLa	3.8
STAT-3	CST	12640S	PVDF	HeLa	3.5
pSTAT-3	CST	9145S	PVDF	HeLa	3.4
ERK2	Genscript	A01194	PVDF	HeLa	17.6
Ikkα	Novus	NB100-56704	PVDF	HeLa	5.9
TRF-2	Novus	NB100-56506	PVDF	HeLa	20.7
GAPDH	Millipore	MAB374	PVDF	HeLa	1.2
Actin	Millipore	MAB1501R	PVDF	HeLa	2.1
Tubulin	Millipore	MAB5564	PVDF	HeLa	3.5
STAT-3	CST	12640S	Nitrocellulose	HeLa	21.0
pSTAT-3	CST	9145S	Nitrocellulose	HeLa	10.6
ERK2	Genscript	A01194	Nitrocellulose	HeLa	4.6
Fibronectin	SAB	38073	Nitrocellulose	Urine	4.2
Albumin	R&D	MAB1455	Nitrocellulose	Urine	1.9
Cytokeratin 18	Novus	NB500-306	Nitrocellulose	Urine	6.9
Myoglobin	SAB	42039	Nitrocellulose	Urine	2.7
Haptoglobin	SAB	23017	Nitrocellulose	Urine	8.6
Fibrinogen	SAB	22542	Nitrocellulose	Urine	1.2
Collagen I	Abcam	ab34710	Nitrocellulose	Urine	3.6
Collagen III	Abcam	ab7778	Nitrocellulose	Urine	4.5
GAPDH	Millipore	MAB374	Nitrocellulose	Jurkat	1.1
STAT-1	Millipore	06-501	Nitrocellulose	Jurkat	3.6
Tubulin	Millipore	MAB1864	Nitrocellulose	Jurkat	12.4
Actin	Millipore	MAB1501R	Nitrocellulose	Jurkat	104.1
STAT-1	Santa Cruz Bio	SC-346	Nitrocellulose	HeLa	1.5
pSTAT-1	CST	7649L	Nitrocellulose	HeLa	4.9
				Median	**4.1**

Table 1. Representative data comparing AdvanBlock-Chemi to 5% Milk-PBST. Various antibodies were used to demonstrate that AdvanBlock-Chemi consistently yields improved performance relative to 5% Milk-PBST regardless of sample type or membrane used without optimization.

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Save on precious antibody

Reuse your antibody up to 4 times when diluted in AdvanBlock-Chemi.

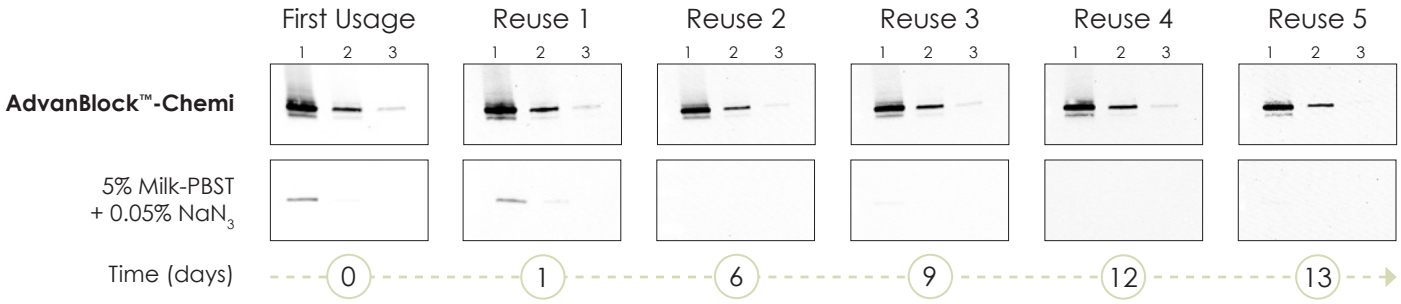

Figure 4. Anti-phospho STAT3 demonstrates increased stability in AdvanBlock-Chemi compared to 5% Milk-PBST + 0.05% NaN_3. (A) 5-fold serial dilutions of IFNα-treated HeLa lysate (5, 1 and 0.2 µg) were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The blots were blocked with AdvanBlock-Chemi or 5% Milk PBST before incubation with anti-phospho STAT-3 (Cell Signaling Technology #9145S). Sodium azide was added to the milk solution used to dilute the primary antibody to prevent microbial growth. After the primary antibody incubation step was complete, each blocking solution was transferred to a sterile 15 mL conical tube and stored at 4°C. Signal was detected with WesternBright ECL. In addition to the enhancement of specific signal, the antibody diluted in AdvanBlock-Chemi shows increased stability demonstrated by the reuse of the antibody solution more than 4 times over the course of two weeks.

Customer feedback

The Advansta AdvanBlock-Chemi reduced the nonspecific background… It was simple to use, straight from the bottle and I would recommend using this product if nonspecific bands are a problem. – **T.U., University of Connecticut**

I have used AdvanBlock-Chemi solution for my experiment, it gives really good signal without background… Compare to using 5% milk, at least 3 times better band signal shows up. – **S.K., Ph.D., Indiana University**

I tested two antibodies. SCBT's monoclonal anti-RWDD4 (C5) and VWR's rabbit antisera anti-GAPDH. Both were diluted 1:1000. AdvanBlock-Chemi was compared to the antibodies diluted in 5% blotting buffer (Bio-Rad) which were tested as per standard protocol... Secondary antibodies were SCBT's goat anti-mouse-HRP (1:3000) and goat anti-rabbit-HRP (1: 6000); these were used at RT for 1 hr… The standard protocol failed to show GAPDH. AdvanBlock-Chemi showed clear GAPDH bands in all samples tested, as well as stronger signal for an artifact band we see when using C5. As blots are worthless without a good positive control signal, we believe that AdvanBlock-Chemi performed better than standard protocols. – **E.H., Touro University California**

Ordering Information

Catalog Number	Product	Size
R-03726-E10	AdvanBlock™-Chemi	1 L

Advansta Corporation
2140 Bering Drive | San Jose, CA 95131
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Product information: www.advansta.com/products/AdvanBlock-Chemi