

Application Solutions for Western Blotting



Western Blotting

Companion Products

Solutions for consistently better blotting.

Companion products from Cell Signaling Technology (CST) are the same reagents used by our scientists when validating our primary antibodies, so we have the experience to support every step of your western blot (WB) or immunoprecipitation (IP) experiment. These products are thoroughly validated and work optimally with our primary antibodies and protocols, so you obtain clean blots with specific signal, for reproducible and publishable results in the least amount of time.

Western blotting success is dependent on the quality of your antibodies and your protocol.

CST provides extensive western blotting reference information from our rigorous in-house testing to support your experiments.

Save time by skipping antibody titrations, because optimal dilutions and dilution buffers are identified for every CST™ antibody. These specifications are generated and supported by testing and are provided on the datasheet that accompanies every antibody.

CST Technical Support

At CST, providing exceptional customer service and technical support are our top priorities. Our scientists work at the bench daily to produce and validate our antibodies, so they have hands-on experience and in-depth knowledge of each antibody's performance. In the process, these same scientists generate valuable reference information that they use to answer your questions and help troubleshoot your experiment by phone or email.

www.cellsignal.com/support (USA & Europe)

www.cst-c.com.cn/support (China)

www.cstj.co.jp/support (Japan)

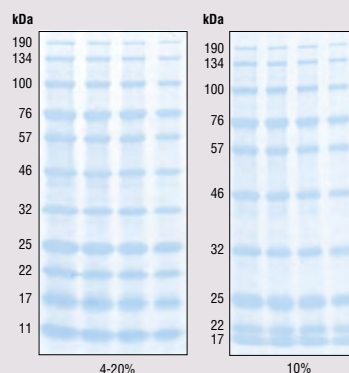


Western Blotting Application Solutions Kit #12957

It takes multiple buffers and reagents to complete your western blot experiment. Save your valuable time by choosing the convenience of the CST Western Blotting Application Solutions Kit, which contains pre-made buffers and reagents to support each step of your protocol.

KIT INCLUDES

- #9803 Cell Lysis Buffer (10X)
- #7722 Blue Loading Buffer Pack
- #13953 Prestained Protein Marker, Broad Range (11-190 kDa)
- #9998 BSA
- #7074 Anti-rabbit IgG, HRP-linked Antibody
- #7076 Anti-mouse IgG, HRP-linked Antibody
- #8553 PMSF
- #4050 Tris-Glycine SDS Running Buffer (10X)
- #12369 Nitrocellulose Sandwiches
- #12539 Tris-Glycine Transfer Buffer (10X)
- #9997 Tris Buffered Saline with Tween® 20 (TBST-10X)
- #9999 Nonfat Dry Milk
- #6883 SignalFire™ ECL Reagent



Prestained Protein Marker, Broad Range (11-190 kDa)
#13953: Titration of Prestained Protein Marker, Broad Range (11-190 kDa) on a 4-20% Tris-glycine gradient SDS-polyacrylamide gel (left) and on a 10% Tris-glycine SDS-polyacrylamide gel (right).

Western Blotting

How important are protease and phosphatase inhibitors?

You Decide.

It takes time and resources to obtain high quality cell extracts for experiments.

Protect the integrity of your samples by always including protease and phosphatase inhibitors in your lysis buffer.

Protease and Phosphatase Inhibitor Cocktails offer the convenience of broad spectrum enzymatic inhibition at an easy to use 100X concentration.

PROTEASE AND PHOSPHATASE INHIBITORS

#5872 Protease/Phosphatase Inhibitor Cocktail (100X)

#5871 Protease Inhibitor Cocktail (100X)

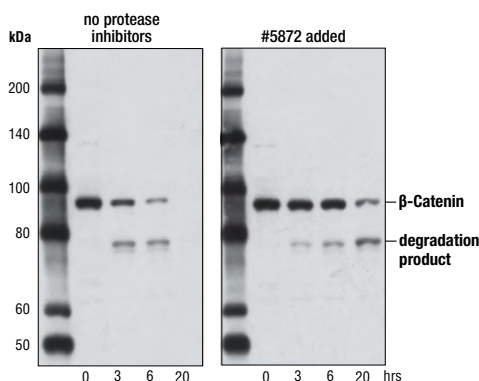
#5870 Phosphatase Inhibitor Cocktail (100X)

#8553 PMSF

Protease Inhibitors Prevent Degradation

Protease/Phosphatase Inhibitor Cocktail (100X) #5872:

WB analysis of NIH/3T3 cell extracts prepared in lysis buffer in the absence of protease inhibitors (left) or with #5872 added (right) was performed at the indicated time points following cell lysis and storage on ice, using β -Catenin (D10A8) XP[®] Rabbit mAb #8480. In the absence of protease inhibitors, β -Catenin signal begins to fade within 3 hr after lysis, indicating protein degradation.

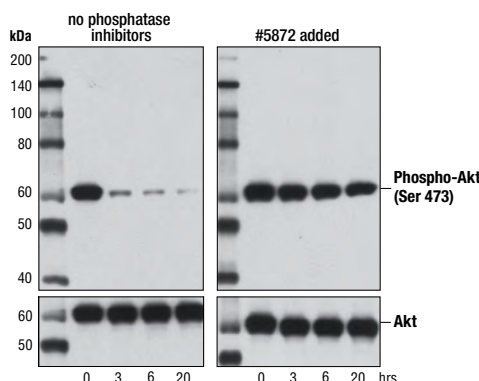


In the presence of the protease inhibitor cocktail, β -Catenin degradation is slowed significantly and signal is still present 20 hr after lysis.

Phosphatase Inhibitors Preserve Phosphorylation

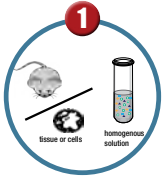
Protease/Phosphatase Inhibitor Cocktail (100X) #5872:

WB analysis of extracts from NIH/3T3 cells, serum-starved overnight and treated with hPDGF-BB #8912 (100 ng/ml, 5 min), prepared in lysis buffer in the absence of phosphatase inhibitors (left) or with #5872 added (right) and was incubated at 37°C for the indicated time following cell lysis, using Phospho-Akt (Ser473) (D9E) XP[®] Rabbit mAb #4060 (upper) or Akt (pan) (C67E7) Rabbit mAb #4691 (lower). In the absence of phosphatase inhibitors, phospho-Akt signal drops significantly after 0 hr, demonstrating rapid loss of phosphorylation at later time points.



In the presence of the phosphatase inhibitor cocktail, phospho-Akt signal is preserved through all monitored time points.

Reliable reagents to support your protocol



SAMPLE PREPARATION

- #7780 BCA Protein Assay Kit
- *#9803 Cell Lysis Buffer (10X)

All CST™ lysis buffers contain protease and phosphatase inhibitors. We recommend adding 1 mM PMSF immediately before use.

TIP: Use #7780 with #9803 to reliably quantify the protein concentration of your samples for more accurate loading.



SDS-PAGE

- #4050 Tris-Glycine SDS Running Buffer (10X)
- #7722 Blue Loading Buffer Pack
- #7727 Biotinylated Protein Ladder Detection Pack (9–200 kDa)
- #13953 Prestained Protein Marker, Broad Range (11–190 kDa)



WET TRANSFER

- #12539 Tris-Glycine Transfer Buffer (10X)
- #12369 Nitrocellulose Sandwiches



BLOCKING

- #9997 Tris Buffered Saline with Tween® 20 (TBST-10X)
- #9999 Nonfat Dry Milk



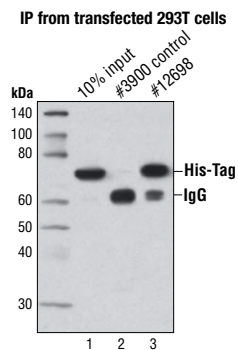
PRIMARY Ab INCUBATION

- #9997 Tris Buffered Saline with Tween® 20 (TBST-10X)
- #9999 Nonfat Dry Milk
- #9998 BSA

Cell Lysis Buffer (10X) #9803

is ideal for lysate concentration determination and IP experiments because it lyses cells under non-denaturing conditions.

Cell Lysis Buffer (10X) #9803: IP of His-Tag protein from transfected 293T cells using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or His-Tag (D3I10) XP® Rabbit mAb #12698 (lane 3). Lane 1 is 10% input. WB analysis was performed using #12698. Cell lysates were prepared using #9803.



Blue Loading Buffer Pack #7722

allows easy observation of sample progression through the gel matrix. Blue Loading Buffer Pack contains 3X SDS Loading Buffer and 30X DTT reducing agent for use in SDS-PAGE analysis.

Nitrocellulose Sandwiches #12369

provide a convenient alternative to cutting membrane and filter paper by hand and are suitable for most common transfer methods including tank, semi-dry, and vacuum blotting. These membrane sandwiches consist of a 7 x 8.5 cm precut 0.2 µm pore size nitrocellulose membrane and 2 sheets of 0.34 mm thick blotting paper.

WB
tools for
success



WB Application Guide

Helpful tips and explanations to support your WB experiments are available in "A Guide to Successful Western Blotting."

www.cellsignal.com/literature



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WASHING

#9997 Tris Buffered Saline with Tween® 20 (TBST-10X)



7

SECONDARY Ab INCUBATION

#9997 Tris Buffered Saline with Tween® 20 (TBST-10X)

#9999 Nonfat Dry Milk

#7076 Anti-mouse IgG, HRP-linked Antibody

#7074 Anti-rabbit IgG, HRP-linked Antibody



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WASHING

#9997 Tris Buffered Saline with Tween® 20 (TBST-10X)



9

DETECTION

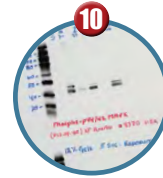
#6883 SignalFire™ ECL Reagent

#12630 SignalFire™ Plus ECL Reagent

#12757 SignalFire™ Elite ECL Reagent

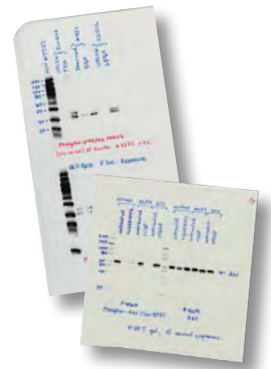
#5571 Anti-biotin (D5A7) Rabbit mAb (HRP Conjugate)

#7075 Anti-biotin, HRP-linked Antibody



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IMAGING

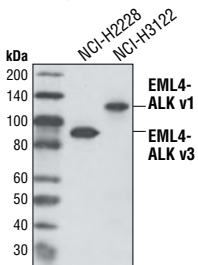


Anti-rabbit IgG, HRP-linked Antibody #7074

yields high sensitivity with short reaction times. Designed for use with rabbit polyclonal and monoclonal antibodies, this affinity purified goat anti-rabbit IgG (heavy and light chain) antibody is conjugated to horseradish peroxidase for chemiluminescent detection.

Anti-rabbit IgG, HRP-linked Antibody #7074: WB analysis of extracts from human non-small cell lung carcinoma cell lines NCI-H2228 and NCI-H3122, using ALK (D5F3) XP® Rabbit mAb #3633 and #7074 (incubated for 1 hr at room temperature). Variants denoting fusions of different EML4 exons (v1 or v3) are indicated.

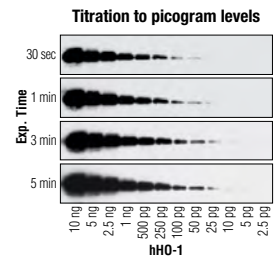
Low background detection of splice variants



SignalFire™ ECL Reagent #6883

provides a more robust signal and extended duration of signal output than other luminol-based systems. SignalFire™ ECL Reagent is an enhanced luminol-based system and capable of detecting low picogram amounts protein.

SignalFire™ ECL Reagent #6883: WB analysis of recombinant human HO-1 (hHO-1) using HO-1 (D60G11) Rabbit mAb #5853 and chemiluminescent detection reagent #6883.



Controls Table

CST has compiled a table of positive and negative controls for many of our antibody targets, so you can be confident your protocol and reagents are working as expected. To view our online controls table so you can make your own control extracts visit:

www.cellsignal.com/controls

Videos

CST scientists perform about one thousand western blots daily to validate our existing and new antibodies. To watch our western blotting protocol and troubleshooting guide videos visit:

www.cellsignal.com/wbvideos



Cell Signaling
TECHNOLOGY®



Cell Signaling Technology (CST) is a private, family-owned company, founded by scientists and dedicated to providing high quality research tools to the biomedical research community. Our employees operate worldwide from our U.S. headquarters in Massachusetts and our offices in the Netherlands, China, and Japan.

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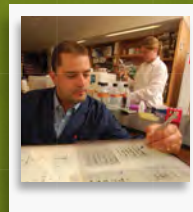
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ORDER INFORMATION

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www.cellsignal.com/orderinfo



FRONT COVER PHOTO:
Andreas, Senior Product Scientist (left), has been at CST since 1999 and Kattie, Sr. Research Associate (right), has been at CST since 2007.

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♻️ Printed on recycled paper (25% post-consumer waste fiber) using vegetable inks and processed chlorine free.

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CST Antibody Performance Guarantee: To learn more, please visit: www.cellsignal.com/abguarantee.

