



Volume 2 Issue 1

WESTERN BLOT

Protein Detection



Sample Preparation



SDS-Page



Wet Transfer



Blocking



Primary Antibody Incubation



Washing



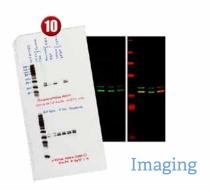
Secondary Antibody Incubation



Washing



Detection





Speed Past the Twist and Turns in Protein Research

Lonza – for Biologically Relevant Results

Novel Precast Gels and Buffers - Fast with High Quality

Lonza has the precast gels, stains and buffers you need. Recently, they introduced their novel PAGEr™ EX and ProSieve™ EX products for fast protein electrophoresis and staining or rapid tank transfer for western blotting. Combined they take standard protein methods down to less than 1 hour.

PAGEr™ EX gels and ProSieve™ buffers

PAGEr™ EX Gels are completely redesigned precast SDS PAGE gels made with faster migration and longer shelf-life formulations, simplifying the full protein separation range offering. This makes choosing the optimal gel easier. The gels are available in cassette sizes compatible with a wide range of chambers.

- All PAGEr™ EX gels have a **1 year shelf life**
- Fast separation 20–25 minutes, with PAGEr™ EX gels and ProSieve™ EX buffers
- Simple selection Separation of proteins 5kDa–350kDa in only 2 range formats
- Razor sharp resolution High quality band intensity

ProSieve™ EX running and transfer buffer system are modified buffer formulations that perform just like tris-glycine, but significantly accelerate run time and transfer time, without compromising results. Complete separation and transfer in just 30 minutes.

- 20 minute run/separation
- 10 minute protein transfer



PAGEr™ EX precast gels

PAGEr™ Gold Precast Gels

Lonza also offers traditional protein electrophoresis products like Laemmli Tris-Glycine gels for polyacrylamide gel electrophoresis (PAGE) along with high quality prepared Tris-Glycine buffer for separation of native proteins and Tris-Glycine-SDS buffer for denatured proteins (SDS PAGE).

The PAGEr™ Minigel Chamber

The PAGEr[™] Minigel Chamber is the easiest chamber you will ever use. The simple, lock-in-place core design creates a tight, flat fit. No need to remove the core, simply insert gels, close the clamps, fill with buffer and run. Runs one or two PAGEr[™] Gels.

- Easy-to-use core eliminates leaking and minimizes handling
- Perfect fit with 9 cm × 10 cm and 10 cm × 10 cm PAGEr[™] Gels
- Even electrical force ensures straight lanes



PAGEr™ EX MInigel Chamber



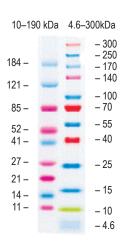
Protein Analysis

ProSieve™ Color Protein Marker, 10–190 kDa

The proteins in the ProSieve™ Color Protein Marker have been labeled with 3 different fluorescent dyes and contains 9 proteins with approximate masses of 10, 15, 20, 25, 40, 50, 80, 125, and 190 kDa.

ProSieve™ QuadColor™ Protein Marker, 4.6–300 kDa

The ProSieve™ QuadColor™ Protein Marker is a mixture of 12 recombinant, highly purified proteins with molecular weights of 4, 6, 10, 15, 25, 40, 55, 70, 100, 140, 170, 250, and 300 kDa. The proteins are individually prestained using four different dyes, producing a brightly colored ladder with an easy-to-remember pattern. The ProSieve™ QuadColor™ Protein Marker is ready-to-use: no heating, further dilution or addition of a reducing agent is required before use.



ProSieve™ Protein Colored Markers

GenScript – Make Research Easy



GenScript ExpressPlus™ PAGE Gels

GenScript ExpressPlus™ PAGE Gels

GenScript ExpressPlus™ PAGE Gels are high-performance precast mini polyacrylamide gels specially designed for large loading volumes. The unique design of the cassette gives better band resolution and improves the sample distribution in the loading wells. GenScript's gel-casting techniques provide excellent batch-to-batch consistency and guarantee a reliable migration pattern.

The ExpressPlus[™] PAGE Gels are available in gradient (4–20%, 4–12%, and 8–16%) and fixed (8%, 10%, and 12%) concentrations and in 10-well, 12-well, and 15-well formats.

- Large loading volume Up to 80 μ l
- Easy to use Wider opening allows sample loading with regular pipette tips
- **High resolution** More even, sharper bands
- Long shelf life Up to 12 months

- Fits most popular mini-gel tanks
- High reproducibility Consistent performance of each gel
- Cost effective



Cell Signaling Technology

WB Validated Antibodies

Western blotting remains one of the most common scientific methods for monitoring protein expression in cells or tissue. The accuracy of western blot results relies heavily of the

quality of the primary antibody employed in the immunoblotting. Cell Signaling Technology provides the highest quality antibodies available for western blotting. CST antibodies are produced in-house and validated extensively according to a rigorous protocol.

Get your free copy of the CST Guide!

CST Pathways

- Chromatin / Epigenetics
- MAP Kinase Signaling
- Apoptosis
- Autophagy Signaling
- PI3K / Akt Signaling
- Calcium, cAMP and Lipid Signaling
- Cell Cycle, Checkpoint Control and DNA Damage
- Cellular Metabolism
- Stem Cell Markers, Development and Differentiation
- Immunology and Inflammation
- Tyrosine Kinase
- Angiogenesis
- Neuroscience

All-in-One Kit – Western Blotting Application Solutions Kit #12957

From sample preparation to detection, everything you need for a more efficient and economical western blot is now found in one easy kit.

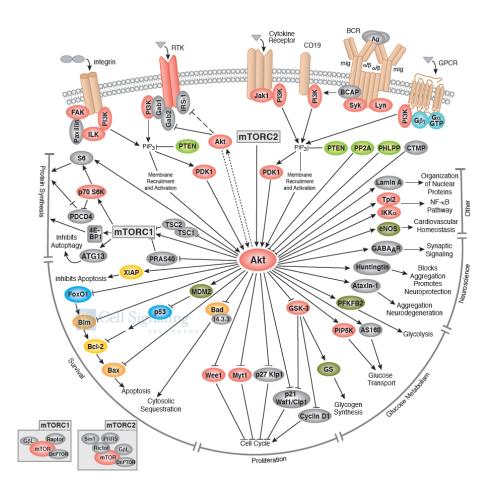


Cell Lysis Buffer (10X) #9803 Blue Loading Buffer Pack #7722 Prestained Protein Marker, Broad Range (11–190 kDa) #7720	
Prestained Protein Marker, Broad Range (11–190 kDa) #7720	
BSA #9998	
Anti-rabbit IgG, HRP-linked Antibody #7074	
Anti-mouse IgG, HRP-linked Antibody #7076	
PMSF #8553	
Tris-Glycine SDS Running Buffer (10X) #4050	
Nitrocellulose Sandwiches #12369	
Tris-Glycine Transfer Buffer (10X) #12539	
Tris Buffered Saline with Tween® 20 (TBST-10X) #9997	
Nonfat Dry Milk #9999	
SignalFire™ ECL Reagent #6883	



Akt Signaling

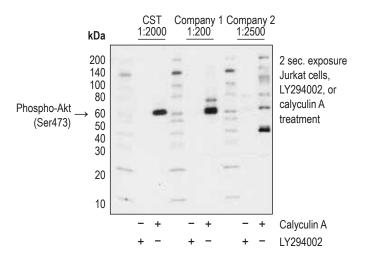
Since its initial discovery as a proto-oncogene, the serine/ threonine kinase Akt (also known as protein kinase B or PKB) has become a major focus of attention because of its critical regulatory role in diverse cellular processes, including cancer progression and insulin metabolism. There are three highly related isoforms of Akt (Akt1, Akt2, and Akt3) and these represent the major signaling arm of PI3K.



Akt Competitor Comparison

Akt is activated by phospholipid binding and phosphorylation within the carboxy terminus at Ser473. The choice of antibody is of major importance to generate accurate results. The specificity of your antibody is fundamental to produce good

research. CST's rigorous validation protocols helps to provide specific and reliable antibodies, which helps you get reproducible and meaningful data every time.



CST's Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060 recognizes a strong specific band at 60 kDa in the Calyculin A-treated (phosphatase inhibitor) Jurkat lysate. The band is not seen after LY294002 (PI3 kinase inhibitor) treatment.

Company 1 phospho-Akt (Ser473) rabbit polyclonal antibody recognized the appropriate band, as well as a cross-reacting band at ~75 kDa. This antibody is not specific for phospho-Akt.

Company 2 phospho-Akt (Ser473) rabbit polyclonal antibody displays significant background banding in addition to the appropriate band, and the band of interest is weaker than other cross-reacting bands.





OriGene – Your Gene Company

TrueMAB™ Monoclonal Antibodies

TrueMAB™ antibodies are superior monoclonal antibodies made **against authentic protein antigens** (mostly full-length proteins expressed in human cell lines) and thus are able to recognize the native epitope(s) of the protein target. This feature makes TrueMAB™ a great tool for immunoassays. A positive control is available at nomimal cost for Every



TrueMAB™ antibody if applicable, which is the HEK293 overexpression lysate of the target protein. This lysate can serve as the positive control in Western blot.

Eurogentec – Custom Antibody Production

Speedy 28-Day

SPEEDY 28-DAY PROTOCOL

The Speedy 28-Day Program

The Speedy 28-day program can be performed in rabbit, rat, guinea pig or goat with the antigen of your choice (peptide, your own protein, or cell).

Eurogentec have been designing peptides for immunizations for over 20 years and have developed specific algorithms for the most accurate design of good antigens. As a result the success rate is >95%.

Speedy-DX

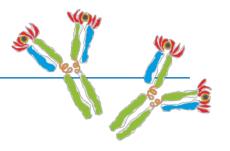
To increase the chances of success the Speedy-DX program uses two peptides, corresponding to spatially distinct regions of the target protein surface. This increases the opportunity of the resultant polyclonals to recognize the target protein in its natural environment.

What is included?

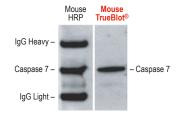
- Peptide design
- Peptide synthesis (> 70%, 15–25 mg, 16 aa)
- Peptide coupling (KLH, BSA, OVA, THY)
- 2 SPF Rabbits, Guinea pigs, Rats or one non-SPF Goat
- 28-day immunization protocol
- All immunizations, blood collections and animal care
- Delivery of the free remaining peptide
- Single shipping on dry ice
- Free ELISA at day 21 for each anti-peptide program



Rockland - Compromise Elsewhere



Immunoprecipitation & Western Blot



TrueBlot®

TrueBlot® preferentially detects the native disulfide form of IgG, making it ideal for IP and Western Blot. It reduces interference by the heavy and light chains of the immunoprecipitating antibody in IP/immunoblotting applications, making data even more accurate.

Revitablot™

Revitablot™ Western Blot Stripping Buffer is faster and more efficient at stripping of primary and secondary antibodies, including HRP and fluorescent labeled. Blots can be stripped multiple times, only incubation of 5–20 minutes needed. It is gentle and effective on both nitrocellulose and PVDF western blot membranes.

Common Secondary Antibodies

- Anti-Rabbit
- Anti-Mouse
- Anti-Goat
- Anti-Donkey
- Anti-Chicken
- Anti-Monkey

LI-COR - Impacting Lives Through Science

The LI-COR C-DiGit Blot Scanner

- Everything You Love about Film, without the Hassle
 - No multiple exposures
 - No saturation
 - No developer
 - No darkroom
- No mess
- No separate analysis
- Lower expense

LI-COR

Do More with Simple, Efficient Data Capture

Increase your productivity by eliminating typical darkroom hassles – like taking multiple exposures or waiting in line. Instead, get the full story in minutes with a single scan.





See More from a Single Scan

Find out what's really happening in highly abundant samples by revealing key differences in strong signals. See both strong and faint bands together in a single digital file.



Discover More with Better Technology

Leave blurred bands and saturation behind. Capture true detail and complexity from every Western blot, due to advancements in optical design and engineered efficiency.



Science where \square

