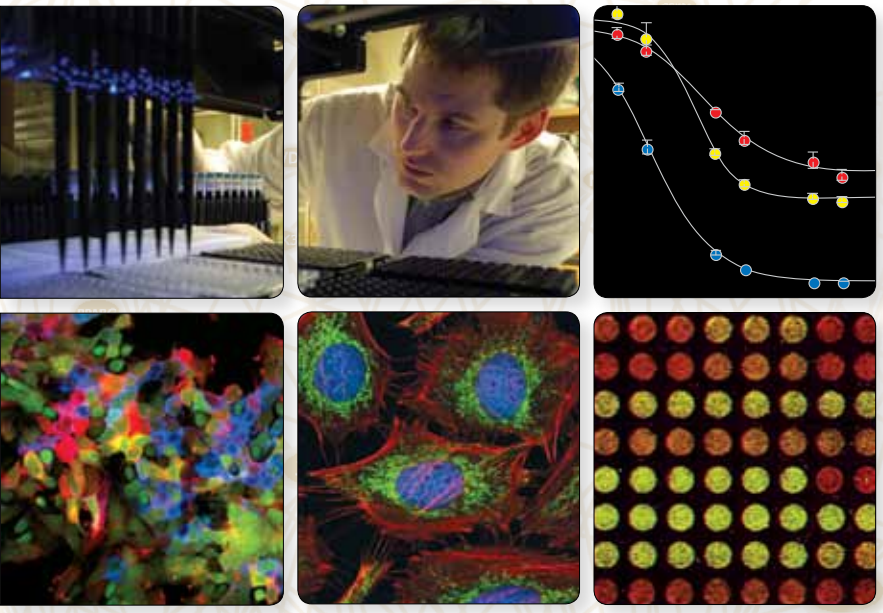


Antibodies and Kits for

Cell-based Screening Assays

Cell Signaling TECHNOLOGY®

XP® Monoclonal Antibodies
for Cell-based Screening Assays

XP® monoclonal antibodies are a line of high quality rabbit monoclonal antibodies exclusively available from Cell Signaling Technology (CST). Any product labeled with XP has been carefully selected based on superior performance in all approved applications.

XP monoclonal antibodies are generated using XMT® technology, a proprietary monoclonal method developed at CST. This technology provides access to a broad range of antibody-producing B cells unattainable with traditional monoclonal technologies, allowing more comprehensive screening and the identification of XP monoclonal antibodies with:

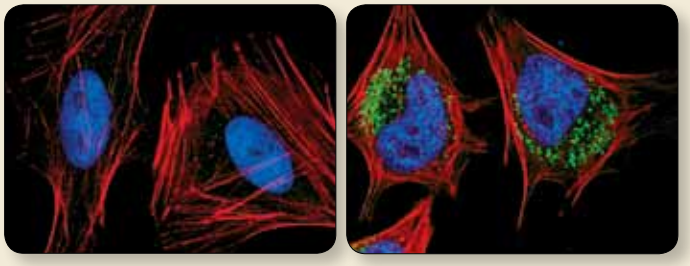
eXceptional specificity
As with all CST™ antibodies, the antibody is specific to your target of interest, saving you valuable time and resources.

+ eXceptional sensitivity
The antibody will provide a stronger signal for your target protein in cells and tissues, allowing you to monitor expression of low levels of endogenous proteins, saving you valuable materials.

+ eXceptional stability and reproducibility
XMT technology combined with our stringent quality control ensures maximum lot-to-lot consistency and the most reproducible results.

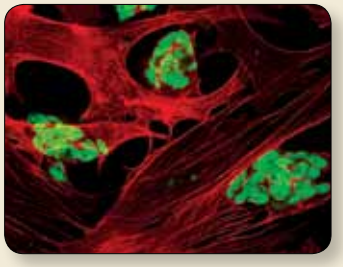
= eXceptional Performance™
XMT technology coupled with our extensive antibody validation and stringent quality control delivers XP monoclonal antibodies with eXceptional Performance in the widest range of applications.

Autophagy



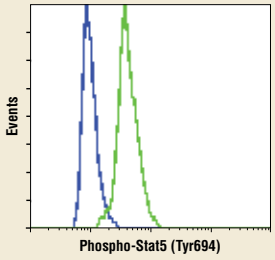
LC3B (D11) XP® Rabbit mAb #3868: *Confocal IF analysis of HeLa cells, untreated (left) or chloroquine-treated (right), using #3868 (green). Actin filaments were labeled using DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).*

Epigenetics



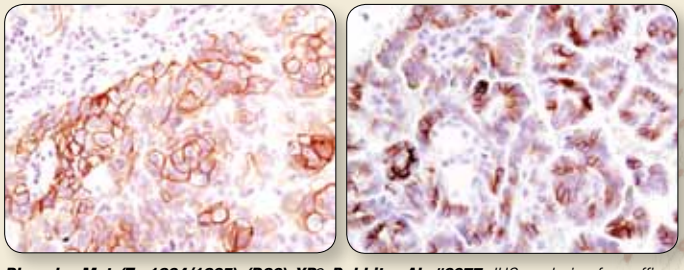
SUZ12 (D39F6) XP® Rabbit mAb #3737: *Confocal iF analysis of mouse embryonic stem cells growing on mouse embryonic fibroblast (MEF) feeder cells using #3737 (green). Actin filaments were labeled with DY-554 phalloidin (red).*

Jak/Stat Signaling



Phospho-Stat5 (Tyr694) (D47E7) XP® Rabbit mAb #4322: *Flow cytometric analysis of TF-1 cells, untreated (blue) or treated with Human Granulocyte Macrophage Colony Stimulating Factor (hGM-CSF) #8922 (green), using #4322.*

RTK Signaling



Phospho-Met (Tyr1234/1235) (D26) XP® Rabbit mAb #3077: *IHC analysis of paraffin-embedded HCC827 xenograft (left) and paraffin-embedded papillary renal cell carcinoma (right) using #3077.*

Visit our website for more experimental details, additional information, and a complete list of available XP® monoclonal antibodies.

WINNER! Life Science Industry Awards®

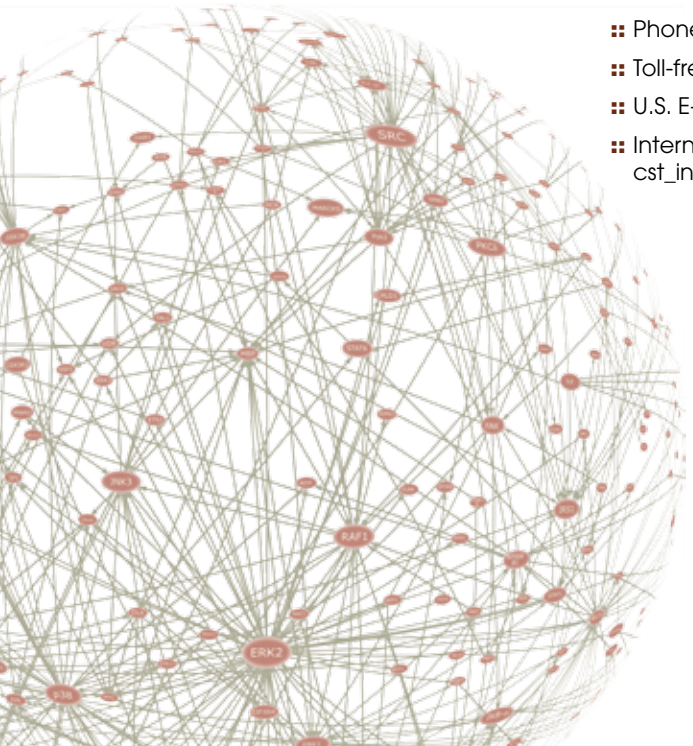
:: *Best Antibodies* :: *Best Breakthrough Products for Cancer Research*

Cell Signaling Technology is the leading provider of signal transduction reagents for the academic and drug discovery communities. Phosphorylation-specific antibodies from CST are the most highly cited in PubMed and are core reagents for multiple drug discovery platforms. We offer a complete line of products to support your cell-based assays at any stage of the drug discovery process. Our high quality reagents have been validated on a large number of popular screening and assay platforms and can be used in most biochemical or cell-based assays. Moreover, our exclusive line of XP® monoclonal antibodies are especially suited for drug discovery applications where exceptional specificity, sensitivity, and reproducibility are of utmost importance.

Find out more:

All CST™ reagents are readily available for testing, lot reservation, custom lot production, and bulk purchases for high throughput screening campaigns.

- Please visit www.cellsignal.com/ddt/custom_reagents.html for further information.
- Contact sales@cellsignal.com for a list of carrier-free formulation antibodies.
- Custom Product Scientists are available to answer technical questions relating to your assay. For assistance, contact support@cellsignal.com.



SALES DEPARTMENT

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- International E-mail: cst_intsales@cellsignal.com

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Environmental commitment: **eco.cellsignal.com**

Custom Reagents

For custom reagents, requests, and services contact sales@cellsignal.com

Custom Formulations

- Carrier-free formulation is available for a broad portfolio of products and is useful for conjugations, custom ELISA development, and other types of customized assay development.

Direct Conjugation Services

- Many of our high quality antibodies are available off-the-shelf already conjugated to Alexa Fluor® 488, 555, 594, and 647 fluorescent dyes, PE, biotin, HRP, or magnetic or sepharose beads.
- Price-competitive and high quality custom antibody conjugation of our catalog antibodies to Alexa Fluor dyes, PE, biotin, HRP, or sepharose or magnetic beads performed upon request.
- Recently revised sizing options available. Contact sales@cellsignal.com for sizing and pricing.

Custom ELISA Production

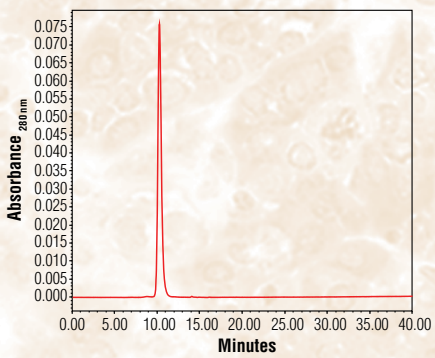
- Custom ELISA products provided in convenient bulk packaging.
- Chemiluminescent and fluorescent detection options available.

Bulk Reservations

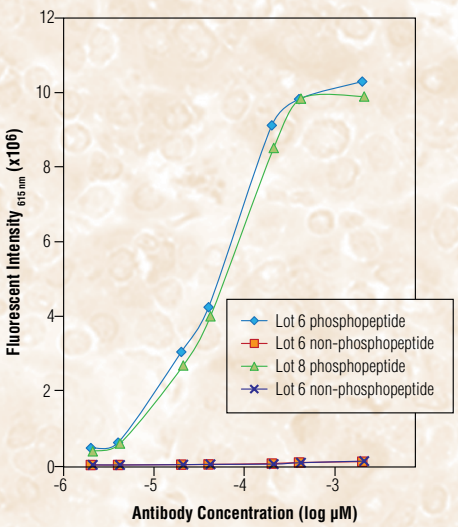
- Single lots of any of our reagents can be reserved for medium or high-throughput assays, such as high content screening, HTRF®-FRET, AlphaScreen®, Meso Scale Discovery®, or other assays.
- Custom bulk quantities are immediately available for most of our antibodies, including all of our high performance XP® monoclonal antibodies.
- Volume-based discounts for carrier-free and all standard glycerol formulations are available upon request.

Custom Product Quality Control

Custom products are held to the same unparalleled standards of quality as off-the-shelf CST™ antibodies. Antibody purity and integrity are assessed by HPLC-SEC, confirming a single population of protein of the correct size, and excluding protein aggregation or degradation. SEC analysis helps ensure the most efficient antibody labeling or spotting. Lot-to-lot consistency is verified in a peptide immunofluorometric assay (DELFIA®) comparing each lot to a previous lot.

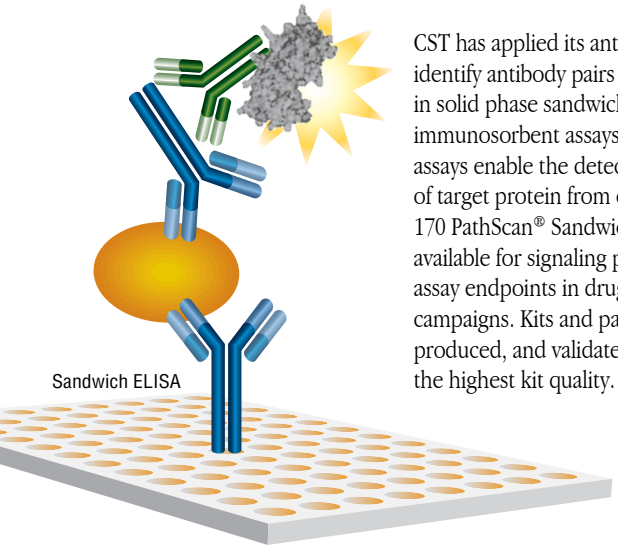


Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb #3377: *HPLC-SEC analysis of a carrier-free lot of #3377 confirms product purity.*



Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb #4858: *Time-resolved immunofluorometric peptide assay was performed with increasing concentrations of two independent lots of #4858 (carrier-free formulation) in the presence of phosphopeptide antigen (blue and green) or the corresponding non-phosphopeptide (red and purple). The assay verifies the phospho-specificity of the antibody and demonstrates consistent performance and minimal lot-to-lot variation.*

PathScan® Sandwich ELISA Kits



CST has applied its antibody expertise to identify antibody pairs with optimal activity in solid phase sandwich enzyme-linked immunosorbent assays (ELISA). These assays enable the detection of low amounts of target protein from cell lysates. Over 170 PathScan® Sandwich ELISA Kits are available for signaling proteins that serve as assay endpoints in drug discovery screening campaigns. Kits and pairs are developed, produced, and validated in-house, ensuring the highest kit quality.

- **PathScan® Sandwich ELISA Kits** contain all necessary components for detection of endogenous levels of key signaling molecules. Matched phospho-specific and total protein ELISA kits are available for many targets. Colorimetric and chemiluminescent detection options available.
- **PathScan® Sandwich ELISA Antibody Pairs** provide scientists with an economical alternative to our complete ELISA kits.
- **PathScan® Multi-Target ELISA Kits** examine several important and well-characterized signaling events in a single assay.
- **PathScan® ELISA Control Cell Extracts** provide the appropriate positive and negative controls and allow the standardization of signal obtained from different plates.
- **Custom ELISA products** allow researchers the option of different detection methods and plate formats. Convenient bulk packaging is available upon request.

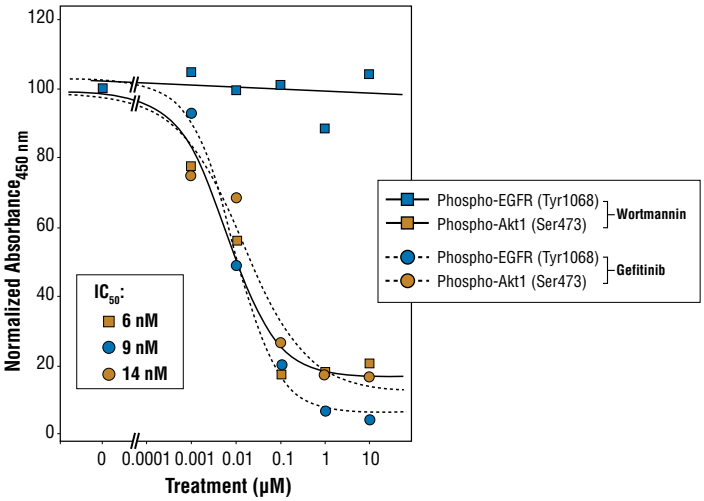
Target	Colorimetric Sandwich ELISA Kits	Chemiluminescent Sandwich ELISA Kits	Antibody Pairs	Multi-Target Kits
Phospho-4E-BP1 (Thr37/Thr46)	#7216		#7854	
4E-BP1	#7179			
Phospho-Acetyl-CoA Carboxylase (Ser79)	#7986			
Acetyl-CoA Carboxlyase	#7996			
β-Actin	#7880		#7881	
Phospho-Akt (Thr308)	#7252	#7135	#7144	Cell Growth #7239
Phospho-Akt1 (Ser473)	#7160	#7134	#7143	Cell Growth #7239 Signaling Nodes #7272
Akt1	#7170	#7132	#7142	Cell Growth #7239 Signaling Nodes #7272
Phospho-Akt2 (Ser474)	#7048			
Phospho-Akt2 (Ser474) (mouse preferred)	#7932			
Akt2	#7046			
Akt2 (mouse preferred)	NEW #7930			
Phospho-Akt3 (Ser472) (mouse preferred)	#7942			
Akt3 (mouse preferred)	#7934			
Phospho-ALK (Tyr1586)	#7159			
Phospho-ALK (Tyr1604)	#7324	#7020		
ALK	#7322	#7084		
Phospho-AMPKα (Thr172)	#7959		#7955	
Phospho-ATF-2 (Thr71)	#7185			
Phospho-Aurora A (Thr288)	#7114		#7115	
Aurora A	#7116		#7117	
Phospho-Axl (panTyr)	#7042			
Axl	#7040			
Phospho-Bad (Ser112)	#7182		#7842	Apoptosis #7105
Bad	#7162		#7840	Apoptosis #7105
E-Cadherin	#7886		#7887	
Cleaved Caspase-3 (Asp175)	#7190			Apoptosis #7105
β-Catenin	#7308		#7309	

Target	Colorimetric Sandwich ELISA Kits	Chemiluminescent Sandwich ELISA Kits	Antibody Pairs	Multi-Target Kits
Phospho-cdc2 (Tyr15)	#7176		#7838	
Phospho-Chk1 (Ser317)	#7870			
Chk1	#7872		#7873	
Phospho-Chk2 (Thr68)	#7037			
Chk2	#7045		#7090	
Phospho-CREB (Ser133)	NEW #7385			
CREB	NEW #7390			
Cox2	NEW #7291			
Phospho-DDR1 (panTyr)	#7863			
DDR1	#7845			
Phospho-EGFR (Tyr845)	#7189			
Phospho-EGFR (Tyr1068)	#7240	NEW #7036		
Phospho-EGFR (Tyr1173)	#7187			
EGFR (E746-A750del Specific)	NEW #8950			
EGFR (L858R Mutant Specific)	NEW #8951			
EGFR	#7250	NEW #7297		
Phospho-eIF2α (Ser51)	#7948			
eIF2α	#7952			
Phospho-eIF4E (Ser209)	#7938			
eIF4E	#7940			
Phospho-eNOS (Ser1177)	#7980			
Phospho-Erk1 (p44 MAPK) (Thr202/Tyr204)	#7315		#7278	
Phospho-Erk1/2 (p44/42 MAPK) (Thr202/Tyr204)	#7177		#7246	Cell Growth #7239 MAP Kinase #7274
Erk1/2 (p44/42 MAPK)	#7050			
Fatty Acid Synthase	NEW #7689			
Phospho-FGFR2 (panTyr)	NEW #7954			
FGFR2	NEW #7939			
Phospho-FLT3 (panTyr)	#7761			

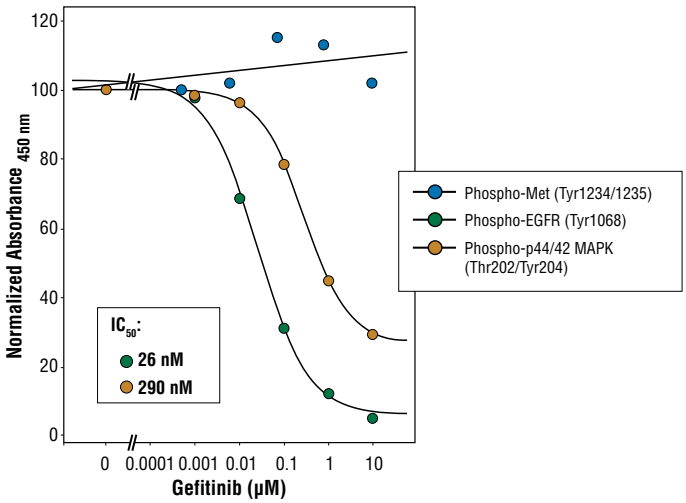
continued>

PathScan® Sandwich ELISA Kits *continued*

Compound titration curves obtained using PathScan® Sandwich ELISA Kits



A-431 cells were exposed to varying concentrations of gefitinib (2 hr) or Wortmannin #9951 (1 hr), then treated with 100 ng/ml Human Epidermal Growth Factor (hEGF) #8916 (5 min). With increasing concentrations of gefitinib, a significant decrease (~25 fold and ~6.25 fold, respectively) in phospho-EGFR and phospho-Akt1 signals were detected using the ***PathScan® Phospho-EGF Receptor (Tyr1068) Sandwich ELISA Kit #7240*** *and the* ***PathScan® Phospho-Akt1 (Ser473) Sandwich ELISA Kit #7160****. With increasing concentrations of wortmannin, a significant decrease (~5 fold) in phospho-Akt1 signal was detected. Wortmannin had no effect on phospho-EGFR levels.*



A-431 cells were exposed to varying concentrations of gefitinib for 2 hr, then treated with a combination of 100 ng/ml Human Epidermal Growth Factor (hEGF) #8916 and 100 ng/ml HGF (5 min). With increasing concentrations of gefitinib, a significant decrease (~20 fold and ~3.5 fold, respectively) in phospho-EGFR and phospho-p44/42 MAPK signals were detected using the ***PathScan® Phospho-EGF Receptor (Tyr1068) Sandwich ELISA Kit #7240*** *and the* ***PathScan® Phospho-p44/42 MAPK (Thr202/Tyr204) Sandwich ELISA Kit #7177****. In contrast, there was no decrease in phospho-Met signal as detected by the* ***PathScan® Phospho-Met (Tyr1234/1235) Sandwich ELISA Kit #7227****.*

Target	Colorimetric Sandwich ELISA Kits	Chemiluminescent Sandwich ELISA Kits	Antibody Pairs	Multi-Target Kits
Phospho-FLT3 (Tyr591)	#7206	#7021		
FLT3	#7202			
GFP	#7878		#7879	
Phospho-HER2 (panTyr)	#7968			
Phospho-HER2 (Tyr1221/1222)	#7148		#7817	
HER2	#7310			
Phospho-HER3 (panTyr)	#7890			
HER3	#7888			
Acetyl-Histone H2A	#7233			
Acetyl-Histone H2B	#7178			
Acetyl-Histone H2B (Lys5)	#7218			
Acetyl-Histone H2B (Lys20)	#7222			
Acetyl-Histone H3	#7232		#7209	
Acetyl-Histone H3 (Lys9)	#7121			
Acetyl-Histone H3 (Lys18)	#7122			
Mono-Methyl Histone H3 (Lys4)	#7123			
Di-Methyl-Histone H3 (Lys4)	#7124			
Tri-Methyl-Histone H3 (Lys4)	#7125			
Pan-Methyl-Histone H3 (Lys9)	#7864			
Di-Methyl-Histone H3 (Lys9)	#7862			
Tri-Methyl-Histone H3 (Lys27)	#7866			
Di-Methyl-Histone H3 (Lys36)	#7868			
Phospho-Histone H3 (Ser10)	#7155	**NEW** #7303	#7207	
Histone H3	#7253			
Acetyl-Histone H4	#7238			
Acetyl-Histone H4 (Lys8)	#7224			
Acetyl-Histone H4 (Lys12)	#7228			
Phospho-HSP27 (Ser78)	#7290			
Phospho-HSP27 (Ser82)	#7152			
HSP27	#7295			
Phospho-IGF-I Receptor β (Tyr1131)	#7302		#7820	
Phospho-IκBα (Ser32)	#7355		#7343	Inflammation #7276
IκBα	#7360		#7831	
Phospho-IKKα (Ser176/180)	#7073			
Total-IKKα	#7078			
Phospho-IKKβ (Ser177/181)	#7080			
iNOS	#7097			
Phospho-Insulin Receptor β (panTyr)	#7082			
Phospho-Insulin Receptor β (Tyr1146)	#7254		#7827	
Phospho-Insulin Receptor β (Tyr1150/1151)	#7258		#7828	
Phospho-Insulin Receptor β (Tyr1345)	#7326		#7823	
Insulin Receptor β	#7069			
Phospho-IRS-1 (panTyr)	#7133		#7347	
Phospho-IRS-1 (Ser302)	#7283		#7284	
Phospho-IRS-1 (Ser307)	#7287		#7288	
Phospho-IRS-1 (Ser612)	#7332			
IRS-1	#7328			
Phospho-IRS-2 (panTyr)	#7860		#7861	

Target	Colorimetric Sandwich ELISA Kits	Chemiluminescent Sandwich ELISA Kits	Antibody Pairs	Multi-Target Kits
IRS-2	#7884		#7885	
Phospho-c-Jun (Ser63)	#7145	#7027	#7141	
c-Jun	#7150	#7028	#7314	
Phospho-c-Kit (panTyr)	#7231		#7294	
Phospho-c-Kit (Tyr719)	#7298		#7299	
c-Kit	#7197			
Phospho-LAT (Tyr191)	#7936		#7937	
Phospho-Lck (Tyr505)	#7941		#7993	
Phospho-MARCKS (Ser152/156)	**NEW** #7251			
MARCKS	**NEW** #7188			
Phospho-MEK1 (Ser217/221)	#7175	#7029	#7211	Signaling Nodes #7272 MAP Kinase #7274
MEK1	#7165	#7030	#7215	MAP Kinase #7274
Phospho-Met (panTyr)	#7333		#7334	
Phospho-Met (Tyr1003)	#7241			
Phospho-Met (Tyr1234/1235)	#7227		#7229	
Phospho-Met (Tyr1349)	#7896			
Met	#7242			
Phospho-NF-κB p65 (Ser536)	#7173		#7834	Inflammation #7276 Signaling Nodes #7272
NF-κB p65	#7174		#7836	Inflammation #7276
Cleaved Notch1 (Val1744)	**NEW** #7194			
Notch1	**NEW** #7245			
p21 Waf1/Cip1	#7167		#7856	
Phospho-p38 (Thr180/Tyr182)	#7946			MAP Kinase #7274 Inflammation #7276 Signaling Nodes #7272
Acetyl-p53	#7236		#7848	
Phospho-p53 (Ser15)	#7365		#7846	Apoptosis #7105
p53	#7370		#7844	Apoptosis #7105
Phospho-p70 S6 Kinase (Thr389)	#7063	#7153	#7053	
p70 S6 Kinase	#7038		#7039	
Phospho-p90 RSK1 (Ser380)	#7965			
p90 RSK1	#7966			
Cleaved PARP (Asp214)	#7262		#7858	Apoptosis #7105
Phospho-PDGFR α/β (panTyr)	**NEW** #7235			
Phospho-PDGFR α (Tyr849)	#7296		#7317	
PDGFR α	#7318		#7264	
Phospho-PDGFR β (Tyr751)	#7345		#7826	
Phospho-PRAS40 (Thr246)	#7327			
PRAS40	**NEW** #7331			
Phospho-PTEN (Ser380)	#7285			
PTEN	#7882		#7883	
Phospho-Ret (panTyr)	#7034			
Ret	#7032			
Phospho-Ros (panTyr)	#7093			
Ros	#7091			
Phospho-S6 Ribosomal Protein (Ser235/236)	#7205	**NEW** #7316	#7201	Cell Growth #7239
S6 Ribosomal Protein	#7225	**NEW** #7337	#7203	Cell Growth #7239
Phospho-SAPK/JNK1/2/3 (Thr183/Tyr185)	#7325	#7849	#7217	MAP Kinase #7274 Inflammation #7276
SAPK/JNK1/2/3	#7330	#7869	#7219	MAP Kinase #7274

Target	Colorimetric Sandwich ELISA Kits	Chemiluminescent Sandwich ELISA Kits	Antibody Pairs	Multi-Target Kits
Phospho-Smad2 (Ser465/467)	#7348			
Smad2	#7244			
Phospho-Src (Tyr416)	#7953		#7963	
Src	#7984		#7992	
Phospho-Stat1 (Tyr701)	#7234			
Phospho-Stat3 (Tyr705)	#7300	#7149	#7146	Signaling Nodes #7272 Inflammation #7276
Phospho-Stat3 (Ser727)	#7995	**NEW** #8026		
Stat3	#7305			
Phospho-Stat5 (Tyr694)	#7113		#7281	
Phospho-Stat6 (Tyr641)		**NEW** #7304		
Phospho-Survivin (Thr34)	**NEW** #7193			
Survivin	#7169			
Phospho-Syk (panTyr)	#7928		#7929	
Phospho-Syk (Tyr525/526)	#7970			
Phospho-mTOR (Ser2448)	**NEW** #7976			
mTOR	**NEW** #7974			
Phospho-TrkA (Tyr490)	#7210			
Phospho-TrkA (Tyr674/675)	#7212			
TrkA	#7208			
Phospho-TrkB (panTyr)	#7108			
Phospho-TrkB (Tyr516)	#7111			
Phospho-TrkB (Tyr706/707)	#7118			
TrkB	#7106			
α-Tubulin	#7944		#7945	
Acetyl-α-Tubulin	#7204			
Phospho-VEGFR-2 (Tyr1175)	#7335		#7824	
VEGFR-2	#7340		#7825	
Phospho-Zap-70 (Tyr319)	#7171		#7852	
Zap-70	#7172		#7850	

Complementary Reagents for PathScan® Sandwich ELISA

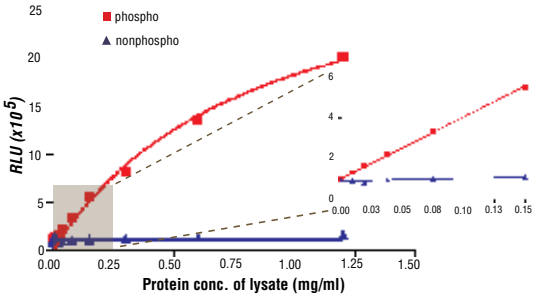
Cell Signaling Technology offers a full set of complementary reagents for ELISA sample preparation and detection.

- #9998 BSA
- #9803 Cell Lysis Buffer (10X)
- #7018 PathScan® Sandwich ELISA Lysis Buffer (1X)
- #9808 Phosphate Buffered Saline (PBS-20X)
- #9809 Phosphate Buffered Saline with Tween 20 (PBST-20X)
- #7002 STOP Solution
- #7004 TMB Substrate

PathScan® Chemiluminescent Sandwich ELISA Kits

PathScan® Chemiluminescent Sandwich ELISA Kits provide the broadest dynamic range and assay sensitivity while requiring half the sample size.

- Chemiluminescent detection system offers broader dynamic range and greater assay sensitivity.
- Low volume microplates retain high signal and sensitivity while using half the sample size of traditional colorimetric ELISA.
- Any standard plate-based luminometer can be used for detection, making specialized equipment unnecessary.
- Technical support is provided by the same scientists that develop and produce the kits, ensuring a thorough, fast, and accurate response.



PathScan® Phospho-ALK (Tyr1604) Chemiluminescent Sandwich ELISA Kit #7020: *Relationship between protein concentrations of lysates from NCI-H2228 cells, lysed with (phospho) and without (nonphospho) the addition of phosphatase inhibitors to the lysis buffer, and immediate light generation with chemiluminescent substrate. Graph inset corresponding to the shaded area shows high sensitivity and a linear response at the low protein concentration range.*

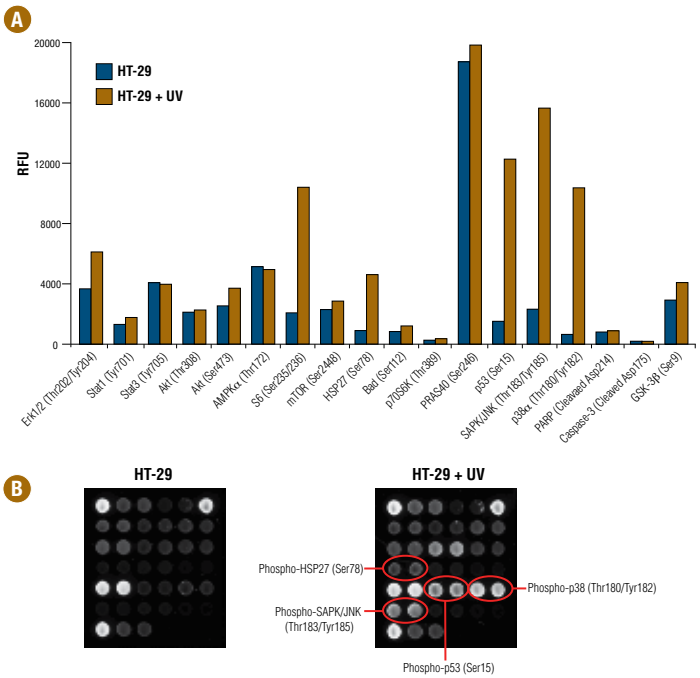
PathScan® Antibody Array Kits

PathScan® Antibody Array Kits provide the opportunity to simultaneously analyze numerous targets with minimal sample requirements.

The kits are slide-based antibody arrays founded on the sandwich immunoassay principle and are designed to investigate signal transduction occuring through key pathway nodes in a multiplex format. These kits include all necessary reagents for either fluorescent or chemiluminescent detection.

- Arrays are developed and optimized in-house, incorporating the highest quality antibodies and ensuring results you can trust.
- Arrays allow the analysis of phosphorylation levels of a large number of proteins per assay, saving valuable time and reagents.
- Arrays are designed to detect RTKs and/or key intracellular molecules, allowing the most comprehensive readout of downstream signaling events.
- Technical support is provided by our in-house Molecular Assay Group, the same scientists who developed and produce the kits.
- The option of chemiluminescent readout allows convenient and easy detection without specialized instrumentation.

	PathScan® Antibody Array Kits	Reactivity
	#7982 PathScan® RTK Signaling Antibody Array Kit (Chemiluminescent Readout)	H
	#7949 PathScan® RTK Signaling Antibody Array Kit (Fluorescent Readout)	H
NEW	#7323 PathScan® Intracellular Signaling Antibody Array Kit (Chemiluminescent Readout)	H, M
NEW	#7744 PathScan® Intracellular Signaling Antibody Array Kit (Fluorescent Readout)	H, M



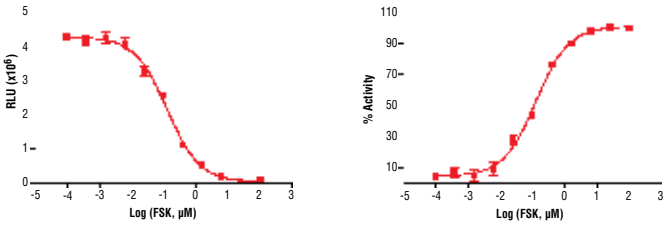
PathScan® Intracellular Signaling Antibody Array Kit (Fluorescent Readout) #7744: *HT-29 cells were grown to 80% confluency, then either left untreated or irradiated with UV for 60 minutes. Lysates were prepared and analyzed using the PathScan® Intracellular Signaling Antibody Array Kit (Fluorescent Readout) #7744. Panel **A** shows quantification of results. Panel **B** shows images that were acquired using the LI-COR® Odyssey® system.*

Cellular Analysis Tools

Cyclic AMP and Cyclic GMP XP® Assay Kits

Cyclic AMP and **Cyclic GMP XP® Assay Kits (#4339 and #4360)** are based on the competition immunoassay principle and can be used to measure the activation of many G protein-coupled receptors (GPCRs). Kits using a chemiluminescent option are also available **(#8019 and #8020)**. In these kits, cyclic nucleotide in the sample of interest competes with a fixed amount of cyclic nucleotide-HRP conjugate provided in the kit for binding to cyclic nucleotide-specific XP® rabbit monoclonal antibody that is precoated on the assay plate. Because of the competitive nature of this assay, the magnitude of the absorbance is inversely proportional to the quantity of cyclic nucleotide in the sample.

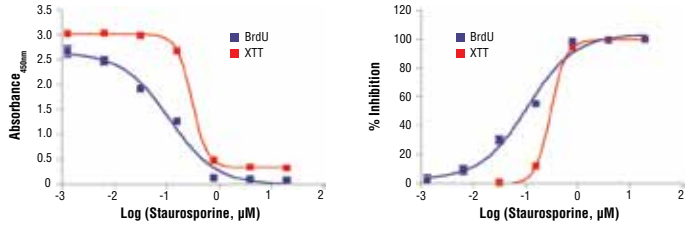
- Highest quality XP monoclonal antibodies employed in the assay ensure the greatest possible sensitivity and specificity.
- Technical support is provided by the scientists who designed and produce these products and know them best.



Cyclic AMP XP® Chemiluminescent Assay Kit #8019: *Treatment of CHO cells with Forskolin (FSK) #3828 increases cAMP concentration as detected by #8019. CHO cells were seeded at 4x10⁴ cells/well in a 96-well plate and incubated overnight. Cells were pretreated with 0.5 mM IBMX (30 min) prior to FSK treatment (15 min) and lysed with 1X Cell Lysis Buffer #9803. The light emission values (left) and percentage of activity (right) are shown. The percentage of activity is calculated as follows: % activity =100X[(RLU-RLU_{basal})/(RLU_{max}-RLU_{basal})], where RLU is the sample relative light unit, RLU_{max} is the light emission at maximum stimulation (i.e. high FSK concentration), and RLU_{basal} is the light emission at basal level (no FSK). FSK directly activates adenylyl cyclases and increases cellular cAMP concentration. IBMX is a non-specific inhibitor of cAMP and cGMP phosphodiesterases and promotes accumulation of cAMP and cGMP in cells.*

NEW XTT Cell Viability Kit

The **XTT Cell Viability Kit #9095** offers a simple means of performing drug discovery compound screens and toxicity studies. In this kit, cellular metabolism is monitored through cellular dehydrogenase-mediated enzymatic reduction of the tetrazolium salt XTT to a colored formazan dye. Metabolically active cells are capable of reducing XTT to a colored dye, and hence, the proportion of dye quantified by measuring absorbance at 450 nm is proportional to the number of viable cells in a sample.

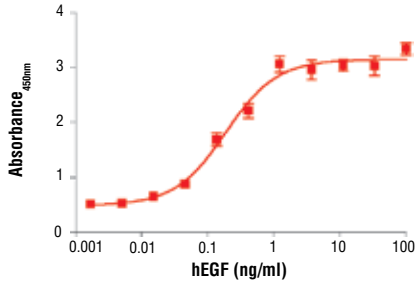


XTT Cell Viability Kit #9095: *HeLa cells were seeded at 1x10⁴ cells/well in a 96-well plate and incubated overnight. Cells were then treated with various concentrations of Staurosporine #9953 overnight. The cytotoxicity was measured using #9095 (red) and* ***BrdU Cell Proliferation Assay Kit #6813*** *(blue) as shown in the left panel. The percent inhibition in each assay was calculated and plotted in the right panel. Staurosporine is a nonspecific kinase inhibitor and induces cellular apoptosis.*

BrdU Cell Proliferation Assay Kit

The **BrdU Cell Proliferation Assay Kit #6813** is a plate-based immunoassay that provides a straight forward means of assaying fundamental cellular activity. This kit offers an accurate, sensitive, and direct readout of cell division unattainable with viability dyes.

- Ability to interface with microplate environment, allowing higher throughput.
- Elimination of the need for microscopy, yielding results without specialized equipment.
- Elimination of the need for radioactive isotope labeling, providing a safer and simpler protocol.



BrdU Cell Proliferation Assay Kit #6813: *Treatment of MCF 10A cells with Human Epidermal Growth Factor (hEGF) #8916 increases cell proliferation as detected by #6813. MCF 10A cells were seeded at 1x10⁴ cells/well in a 96-well plate and incubated overnight. Cells were then starved in serum-free medium overnight. hEGF was added to the plate and cells were incubated for 24 hr. Finally, 10 µM BrdU was added to the plate and cells were incubated for 4 hr.*

Whole Cell Assays

Antibody Validation for Cell-based Immunofluorescent Assays

Scientists at Cell Signaling Technology (CST) have validated over 800 activation state-specific (e.g., phosphorylation-specific) and total protein antibodies for immunofluorescence (IF) applications such as automated cell-based assays.

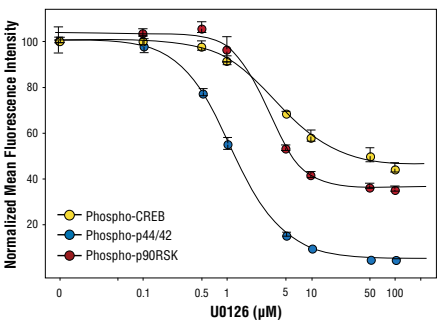
All CST™ antibodies approved for use in fluorescent cell-based assays have undergone a rigorous validation process to ensure optimal performance, including in-house testing on various high content platforms, such as the TTP LabTech Acumen® ^{e}X3 and Cellomics® ArrayScan® V^{TI}. Additionally, our exclusive line of XP® monoclonal antibodies display the exceptional specificity, sensitivity, and reproducibility needed for the most robust automated fluorescent assays.

Please contact our High Content Screening Group at HCS@cellsignal.com for more information or assistance with your HCS assays.

Validation Includes:

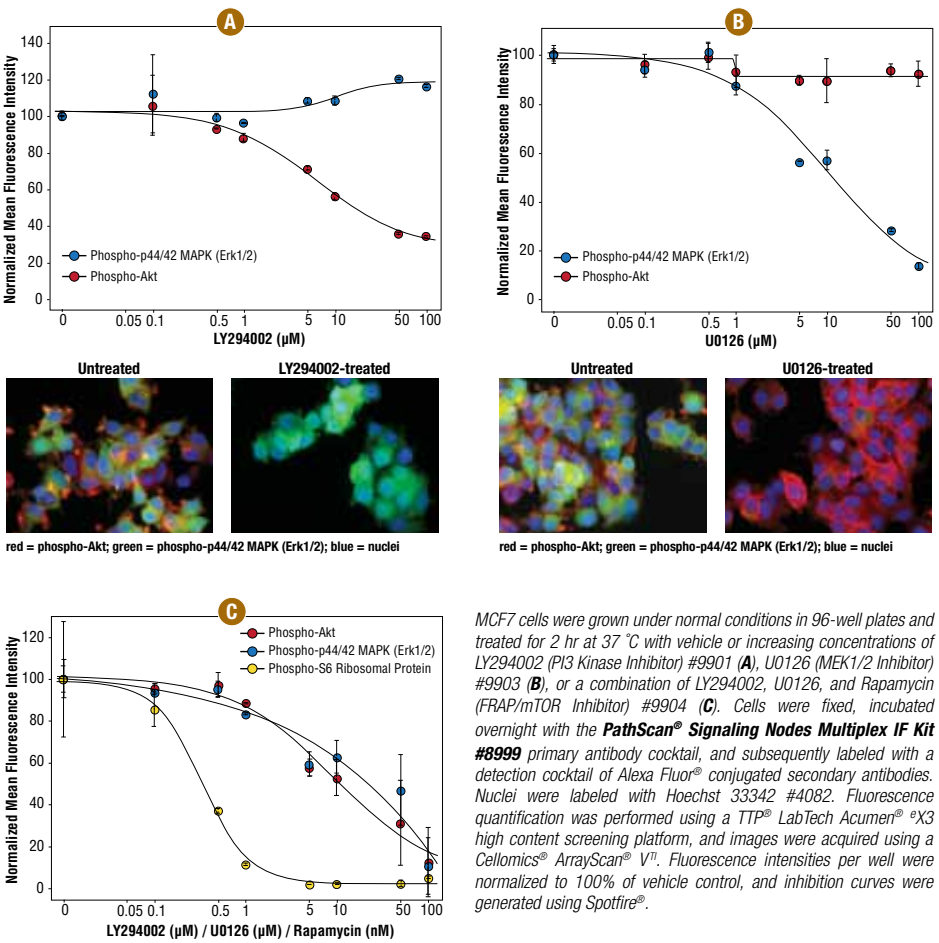
- Antibody titration to determine optimal antibody concentration.
- Treatment of cell lines with pathway-specific inhibitors to verify treatment-induced translocation.
- Phosphatase treatment to verify phospho-specificity.
- Requirement of threshold signal-to-noise ratio in antibody:isotype comparison to ensure greatest possible sensitivity.
- Stringent testing to ensure lot-to-lot consistency.
- Antibody performance demonstrated in-house on multiple high content platforms.

MAP Kinase Signaling



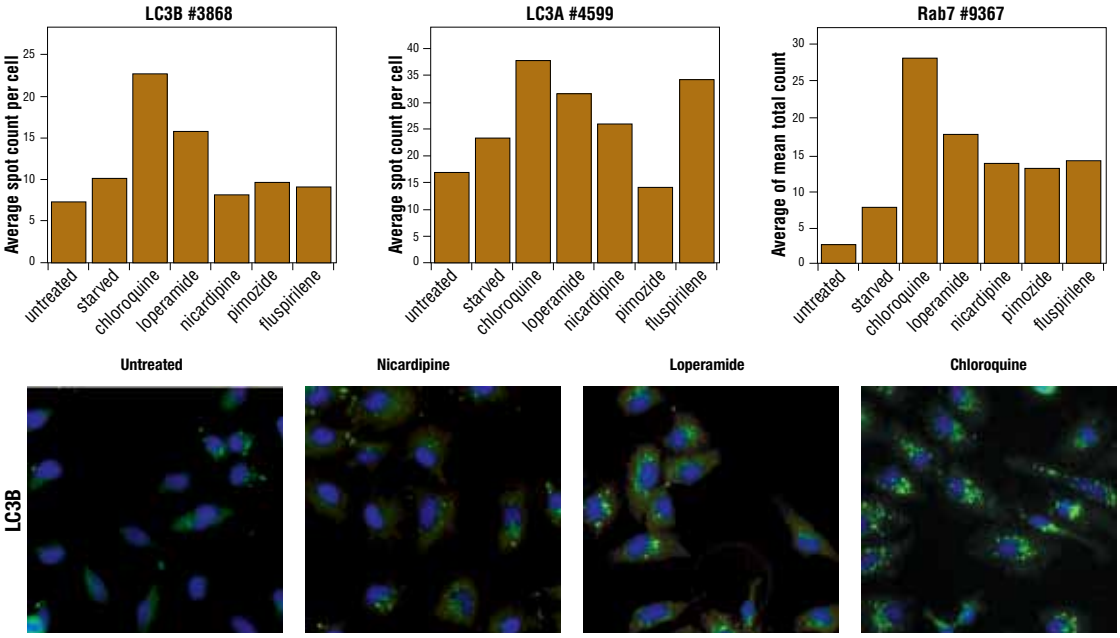
A549 cells were concurrently exposed to U0126 (MEK1/2 Inhibitor) #9903 and the phorbol ester TPA #4174 to assess the effect on MAPK signaling. Phospho-p44/42 MAPK (Erk1/2), detected with ***Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370****, and two downstream signaling proteins, phospho-p90RSK and phospho-CREB, detected with* ***Phospho-p90RSK (Thr573) Antibody #9346*** *and* ***Phospho-CREB (Ser133) (87G3) Rabbit mAb #9198****, were used as readouts. U0126 was titrated as an 8-point dose curve in triplicate to monitor the reproducibility and validity of the assay. The signal for each antibody was analyzed using an Acumen® ^{e}X3 and images were acquired with a Cellomics® ArrayScan® V^{TI}. With increasing concentrations of U0126, a significant decrease in phospho-p44/42 MAPK (Erk1/2) signal (~10-fold), as well as phospho-p90RSK and phospho-CREB (>2-fold), was observed as compared to the TPA-stimulated control.*

Akt/PI3K and MAP Kinase Signaling



*MCF7 cells were grown under normal conditions in 96-well plates and treated for 2 hr at 37 °C with vehicle or increasing concentrations of LY294002 (PI3 Kinase Inhibitor) #9901 (**A**), U0126 (MEK1/2 Inhibitor) #9903 (**B**), or a combination of LY294002, U0126, and Rapamycin (FRAP/mTOR Inhibitor) #9904 (**C**). Cells were fixed, incubated overnight with the* ***PathScan® Signaling Nodes Multiplex IF Kit #8999*** *primary antibody cocktail, and subsequently labeled with a detection cocktail of Alexa Fluor® conjugated secondary antibodies. Nuclei were labeled with Hoechst 33342 #4082. Fluorescence quantification was performed using a TTP® LabTech Acumen® ^{e}X3 high content screening platform, and images were acquired using a Cellomics® ArrayScan® V^{TI}. Fluorescence intensities per well were normalized to 100% of vehicle control, and inhibition curves were generated using Spotfire®.*

Autophagy Markers



HeLa cells were subjected to various autophagy-inducing treatments as labeled (8 hr of serum starvation or the following drugs for 2 hr: chloroquine 50 µM, nicardipine 4.8 µM, loperamide 4.9 µM, pimozide 5.4 µM, or fluspirilene 5.3 µM) and autophagy levels were assessed using three markers, detected using ***LC3B (D11) XP® Rabbit mAb #3868, LC3A (D50G8) XP® Rabbit mAb #4599,*** *and* ***Rab7 (D95F2) XP® Rabbit mAb #9367****. The signal for each antibody was analyzed using an Acumen® eX3 and images for LC3B were acquired with a Cellomics® ArrayScan® VTI.*

PathScan® Multiplex IF Kits

PathScan® Multiplex IF Kits provide a novel multiplex assay for use with automated imaging or laser scanning high content platforms or manual immunofluorescence microscopy. The kits contain a cocktail of three high quality primary antibodies as well as a detection cocktail utilizing the Alexa Fluor® series of fluorescent dyes. Antibody and dye pairings have been pre-optimized, and each kit contains enough reagents for 100 assays (based on a working volume of 100 µl/test).

- Kits allow the analysis of multiple pathway endpoints within a single sample, saving time and reagents.
- Kits are produced and optimized in-house with the highest quality antibodies, providing you with the greatest possible specificity and sensitivity.
- Technical support is provided by our in-house IF group who developed the products and know them best.

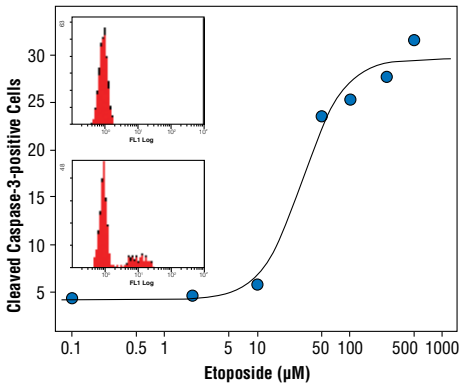
PathScan® Multiplex IF Kits (100 assays)	Reactivity
#7967 PathScan® EGF Receptor Activation Multiplex IF Kit Kit includes: cocktails to simultaneously detect levels of EGF receptor, phospho-EGF receptor (Tyr1068), phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	H, Mk, (M)
#8999 PathScan® Signaling Nodes Multiplex IF Kit Kit includes: cocktails to simultaneously detect levels of phospho-Akt (Ser473), phospho-p44/42 MAPK Erk1/2 (Thr202/Tyr204), phospho-S6 ribosomal protein (Ser235/236)	H, M, R, Mk
#7851 PathScan® Apoptosis and Proliferation Multiplex IF Kit Kit includes: cocktails to simultaneously detect levels of phospho-histone H3 (Ser10), cleaved PARP (Asp214), α-tubulin	H, Mk

Antibody Validation for Flow Cytometry

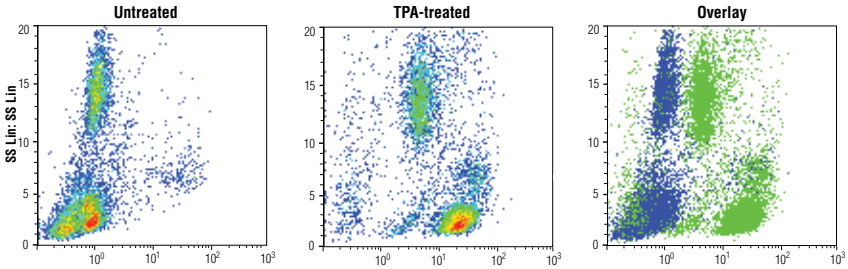
Over 550 Cell Signaling Technology (CST) antibodies have been validated by our in-house Flow Cytometry Group – our goal is to provide our customers with the most specific antibodies, brightest signal, and lowest background possible. CST also offers Alexa Fluor® conjugates and phycoerythrin (PE) conjugates optimized for flow cytometry. These reagents allow flow cytometry to be applied as a platform for the analysis of disease mechanisms, biomarker identification, and drug discovery.

Validation Includes:

- Titration to determine optimal dilution.
- Comparison of signal to isotype control to estimate the nonspecific binding of primary antibodies.
- Treatment of cell lines with pathway-specific inhibitors to verify target specificity.
- Use of known positive and negative cell lines to verify target specificity.
- Phosphatase treatment to confirm phospho-specificity of the antibody.
- Extensive quality control testing to guarantee stability over time and ensure lot-to-lot consistency.



Cleaved Caspase-3 (Asp175) Antibody (Alexa Fluor® 488 Conjugate) #9669: *Jurkat cells were exposed to varying concentrations of etoposide for 6 hours. Increasing concentrations resulted in a greater percentage of cleaved caspase-3-positive cells as detected using #9669. Inset: Flow cytometric analysis of Jurkat cells, untreated (upper) or etoposide-treated (500 μM, lower), using #9669.*

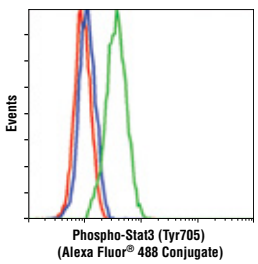


Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #4803: *Flow cytometric analysis of whole blood, untreated or treated with TPA #4174, using #4803.*

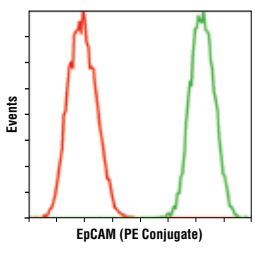
Conjugated Antibodies

We offer a growing selection of our best performing antibodies conjugated to Alexa Fluor® dyes, biotin, PE, HRP, and sepharose or magnetic beads. Antibody conjugation is performed in-house, and is followed by rigorous quality control and stringent validation protocols. Technical support is provided by the same scientists who produce the products and know them best.

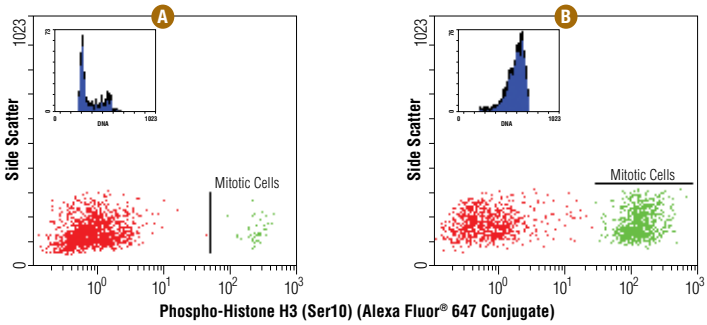
- **Alexa Fluor® 488, 555, 594, and 647 conjugates** – most ideal for highly sensitive immunofluorescent and flow cytometric assays
- **PE conjugates** – optimal for multiplexing with other labeled antibodies in flow cytometry
- **Bead conjugates** – covalently immobilized on sepharose or magnetic beads for simple and efficient immunoprecipitation
- **Biotinylated antibodies** – for use with streptavidin detection systems
- **HRP conjugates** – for one-step western blot detection
- **Custom formulations** – available upon request (see page 4)



Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #4323: *Flow cytometric analysis of Jurkat cells, untreated (blue) or treated with Human Interferon-α1 (hIFN-α1) #8927 (green), using #4323 compared to concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 488 Conjugate) #2975 (red).*



EpCAM (VU1D9) Mouse mAb (PE Conjugate) #8995: *Flow cytometric analysis of HT-29 cells using #8995 (green) compared to concentration-matched Mouse (G3A1) mAb IgG1 Isotype Control (PE Conjugate) #6899 (red).*



Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) #3458: *Flow cytometric analysis of Jurkat cells, untreated (**A**) or taxol-treated (**B**), using #3458. The blue inserts represent PI (DNA) staining alone, showing an increase in the number of mitotic cells in the taxol-treated sample.*

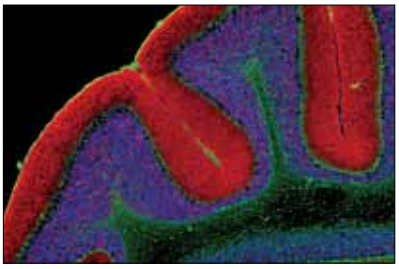
Complementary Reagents for Whole Cell Assays

Along with the highest quality primary antibodies for immunofluorescence, HCA applications, and flow cytometry, CST offers complementary reagents, including dye-conjugated secondary antibodies, and fluorescent DNA dyes for your convenience.

Alexa Fluor® Conjugated Secondary Antibodies

- Alexa Fluor dyes exhibit superior brightness and photostability, providing you with greater sensitivity and longer image capture time.
- Alexa Fluor conjugated secondary antibodies are F(ab')$_2$ fragments, to minimize background staining.
- These reagents are used in-house for antibody validation in immunofluorescence and flow cytometry applications and work optimally with our primary antibodies.

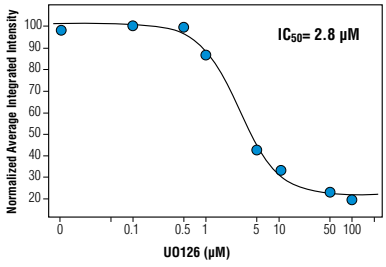
Alexa Fluor® Conjugate	488	555	594	647
Anti-mouse IgG (H+L), F(ab')$_2$ Fragment	#4408	#4409	#9626	#4410
Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment	#4412	#4413	#9679	#4414
Anti-rat IgG (H+L)	#4416	#4417	—	#4418



*Confocal IF analysis of mouse cerebellum using α-Synuclein Antibody (IF Preferred) #2628 detected with **Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 555 Conjugate) #4413** (red) and Neurofilament-L (DA2) Mouse mAb #2835 detected with **Anti-mouse IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4408** (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).*

DyLight® 680 or 800 Conjugated Secondary Antibodies

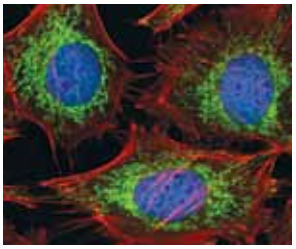
- Near infrared DyLight® dyes avoid autofluorescence commonly observed in green regions of the spectrum, making them ideal for fluorescent western and In-Cell Western™ assays.
- DyLight dyes exhibit low background and high photostability, providing you with greater sensitivity.
- DyLight conjugated secondary antibodies are tested in-house, and technical support is provided by the scientists who use the dyes and know them best.



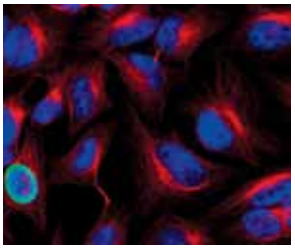
***Anti-Rabbit IgG (H+L) DyLight® 800 Conjugate #5151:** In-Cell Western™ analysis of A549 cells exposed to varying concentrations of U0126 (MEK1/2 Inhibitor) #9903 (3 hr), followed by TPA #4174 stimulation (30 min). With increasing concentrations of U0126, a significant decrease (~5 fold) in the signal from Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370 as compared to the TPA-stimulated control was observed. When using phospho-p44/42 MAPK (Erk1/2) as a measurement, the IC_{50} of this compound was 2.8 µM. Data and images were generated on the LI-COR® Biosciences Odyssey® Infrared Imaging System using #5151 for detection.*

NEW Fluorescent DNA Dyes

DRAQ5® #4084, a cell permeable far-red fluorescent DNA dye, can be used in live or fixed cells. Hoechst 33342 #4082, DAPI #4083, and Propidium Iodide (PI)/RNase Staining Solution #4087 are also available.



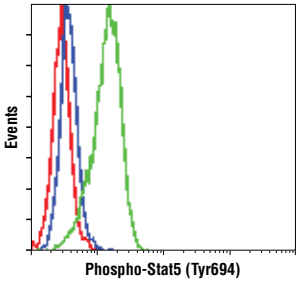
***DRAQ5® #4084**: Confocal IF analysis of HeLa cells using COX IV (3E11) Rabbit mAb #4850 (green). Actin filaments were labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = #4084.*



***Hoechst 33342 #4082:** Confocal IF analysis of HeLa cells using α-Tubulin (DM1A) Mouse mAb #3873 (red) and Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb #3377 (green). Blue pseudocolor = #4082.*

Isotype Controls

Isotype control antibodies are used to estimate the non-specific binding of primary antibodies due to F_c receptor binding or other protein-protein interactions and should have the same immunoglobulin type as the test antibody. Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 is not directed against any known antigen and functions as an isotype control for rabbit IgG monoclonal antibodies. The antibody is also offered conjugated to Alexa Fluor® 488 and 647 (#2975 and #2985).



***Rabbit (DA1E) mAb IgG XP® Isotype Control #3900:** Flow cytometric analysis of K-562 cells, untreated (green) or imatinib-treated (blue), using Phospho-Stat5 (Tyr694) (C71E5) Rabbit mAb #9314 compared to concentration-matched #3900 (red).*

Activators and Inhibitors

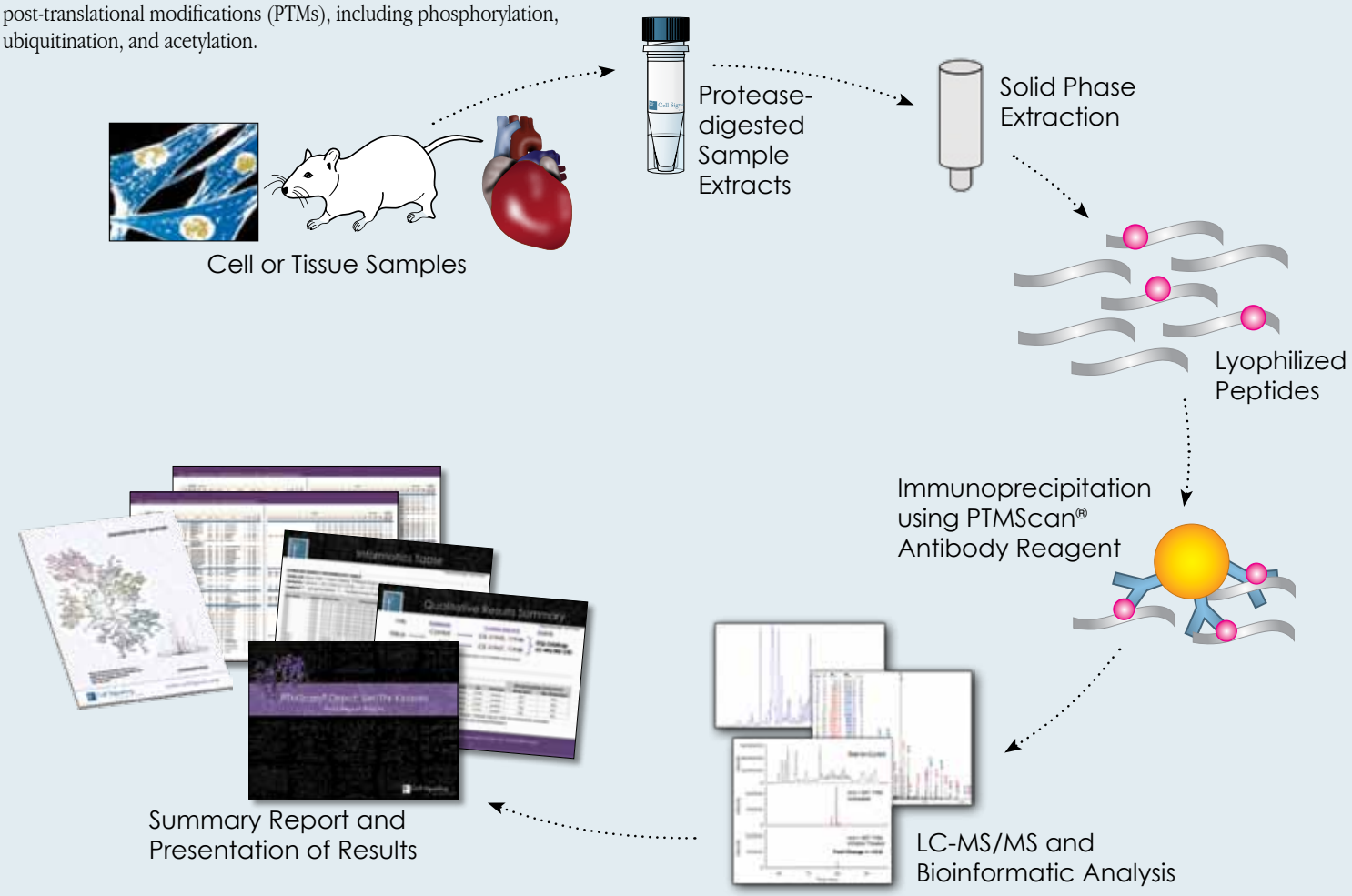
A number of chemical modulators, including U0126, LY294002, Rapamycin, and TPA featured throughout this brochure are also available. Visit our website for a full listing of complementary reagents for whole cell assays.

PTMScan® Proteomics Services

For more information visit www.cellsignal.com/proteomics or contact ptmscan@cellsignal.com

Cell Signaling Technology (CST) employs proprietary methodologies for identifying and quantifying post-translational modifications.

The method uses antibody-based peptide enrichment combined with tandem mass spectrometry for quantitative profiling of post-translational modifications (PTMs), including phosphorylation, ubiquitination, and acetylation.



Available Motif Antibody Kits

A collection of products enables you to use CST™ patented Motif Antibodies:

- **KinomeView® Profiling Kit** provides a western blotting strategy to dissect the complexity of the phosphoproteome.
- **PTMScan® Motif Antibody Kits** are ready to use in studies that employ PTMScan Technology.
- Bulk quantities of PTMScan® Motif Antibody beads are also available. (inquire at ptmscan@cellsignal.com)

AQUA™ Peptides

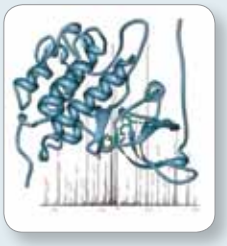
Custom-synthesized, stable isotope-labeled AQUA™ peptides are available to validate and quantify novel findings through PTMScan® Technology.

NOTICE to NOT-FOR-PROFIT or ACADEMIC PURCHASER: Purchase of this peptide product includes a license to use this product in the practice of methods described in United States Patent Application Ser. No. 60/312,279 and PCT US02/25778 (Gygi et al., Harvard University; PROTEIN-AQUA™ methodology/technology) for research purposes only, and the purchase price includes an allowance for payment to Harvard University of its standard royalty for such license. Licenses for the practice of the methods for other non-research purposes may be sought directly from the owner of the technology. NOTICE to FOR-PROFIT PURCHASER: The purchase of this peptide product does not include a license to use this product in the practice of methods described in United States Patent Application Ser. No. 60/312,279 and PCT US02/25778 (Gygi et al., Harvard University; PROTEIN-AQUA™ methodology/technology). Licenses for the practice of such methods must be sought directly from the owner of the technology: Harvard University Office of Technology Licensing Building A, Room 414, 25 Shattuck Street, Boston, MA 02115

We provide the following two types of proteomics services, each uniquely tailored specifically to your experimental needs.

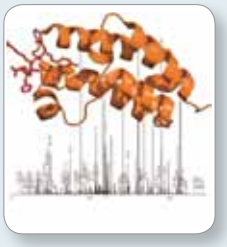
PTMScan® Discovery

A proteomics method for the identification and quantitation of known and novel post-translational modifications (PTMs), including Ser, Thr, and Tyr phosphorylation, as well as ubiquitination and acetylation. The method employs immunoaffinity purification using proprietary motif antibodies developed at Cell Signaling Technology coupled with tandem mass spectometry for comprehensive profiling of up to thousands of PTMs from cell line and tissue samples. This **discovery-mode** proteomics technology is useful for the study of PTMs throughout the proteome in a variety of biological model systems and disease states.



PhosphoScan® Service provides a powerful strategy for kinome-wide phosphoproteomics employing phospho-motif antibodies to analyze the most biologically relevant regions of the kinome.

UbiScan® Service is a sensitive method utilizing a unique ubiquitin-branch antibody for ubiquitinated sequence identification and the most comprehensive profiling analysis of cellular ubiquitin pathways.

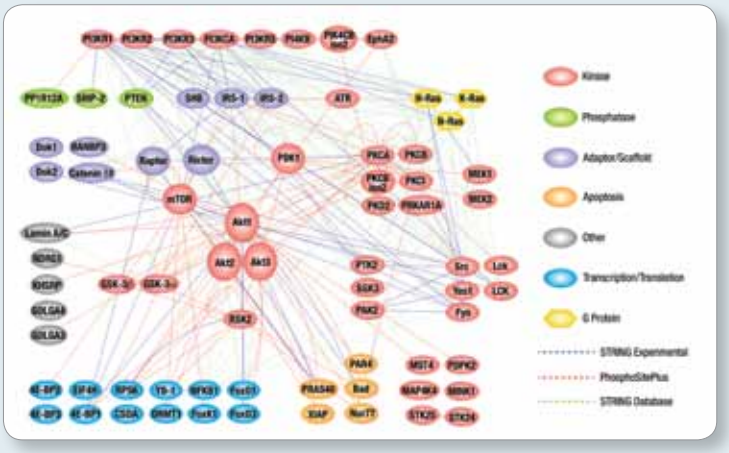


AcetylScan® Service provides comprehensive qualitative and quantitative analysis of protein acetylation using acetylated-lysine antibodies.

PTMScan® Direct

A multiplex proteomics assay for quantitative measurement of a defined set of known post-translational modifications (PTMs) on critical signaling nodes within a group of cellular signaling pathways. The method employs an immunoaffinity purification technique using proprietary modification state-specific antibodies coupled with mass spectrometry. The method allows for targeted screening of hundreds of defined signaling proteins from cell line and tissue samples. This **targeted-mode** proteomics technology is used to investigate changes in PTMs to specific protein targets in response to a drug treatment, disease state, or other biological process of interest.

- **PTMScan® Direct Ser/Thr Kinases Service** to assess the activation state of a defined set of Ser/Thr kinases (385 unique phosphorylation sites to 130 proteins).
- **PTMScan® Direct Tyrosine Kinases Service** to assess the activation state of a defined set of Tyr kinases (671 unique phosphorylation sites to 120 proteins).
- **PTMScan® Direct Multi-pathway Service** to study a defined set of phosphorylated proteins spanning key signaling nodes associated with cancer and disease (111 unique phosphorylation sites to 56 proteins).
- **PTMScan® Direct PI3K/Akt Signaling Service** to perform analysis of a defined set of activated proteins in the PI3K/Akt Signaling pathway (296 unique phosphorylation sites to 105 proteins).



Connectivity between nodes detected in the PTMScan® Direct PI3K/Akt Signaling Service.

Cytokines and Growth Factors

The world's highest quality antibody provider has now extended its expertise to cytokine and growth factor production.

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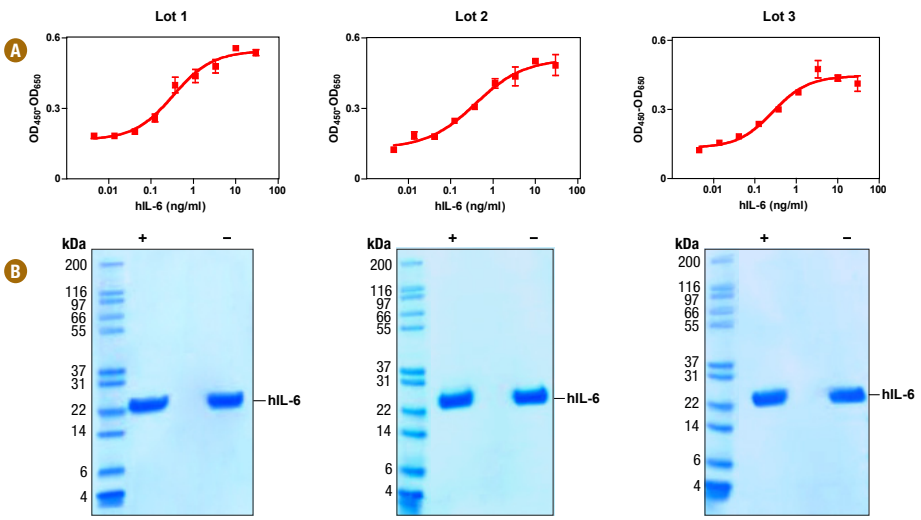
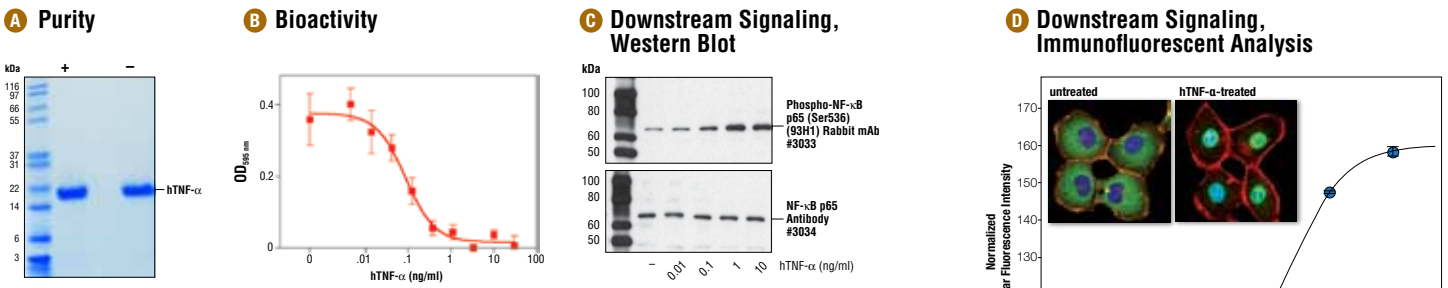
Lot-to-Lot Consistency



Comparison of purity and bioactivity of three independent lots of Human Interleukin-6 (hIL-6) #8904: *The proliferation of TF-1 cells was assessed after 48 hr treatment with increasing concentrations of hIL-6. Cells were incubated with a tetrazolium salt and the OD_{450} - OD_{650} was determined (**A**). The purity of recombinant hIL-6 was determined by SDS-PAGE using 6 µg reduced (+) and non-reduced (-) recombinant hIL-6 and staining overnight with Coomassie Blue (**B**).*

Human Tumor Necrosis Factor-α (hTNF-α) #8902 validation includes:



Human Tumor Necrosis Factor-α (hTNF-α) #8902: *(**A**) 6 µg reduced (+) and non-reduced (-) hTNF-α; hTNF-α does not contain tags or additional amino acids. (**B**) The viability of L-929 cells treated with increasing amounts of hTNF-α in the presence of 2 ng/ml actinomycin D was determined. (**C**) Phosphorylation of NF-κB p65 was measured in response to treatment with hTNF-α. (**D**) HT-1080 cells were treated with increasing concentrations of hTNF-α to assess the effect on nuclear translocation of NF-κB, detected using NF-κB p65 (D14E12) XP® Rabbit mAb #8242. All data points were performed in triplicate to ensure validity and reproducibility of the assay. The signal was analyzed using an Cellomics ArrayScan® VTI. Inset: Confocal IF analysis of HT-1080 cells, untreated (left) or treated with hTNF-α (20 ng/ml, 20 min) (right), using NF-κB p65 (D14E12) XP® Rabbit mAb #8242 (green). Red = Actin filaments (DY-554 phalloidin); Blue = DRAQ5® #4084 (DNA dye).*

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