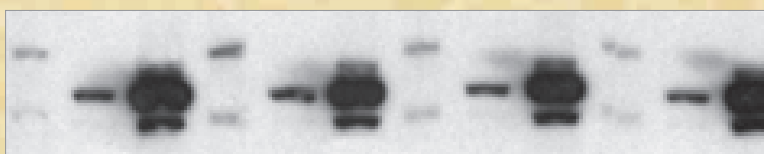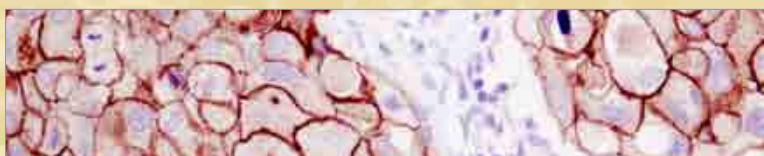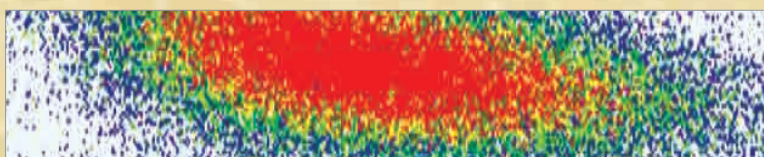# XP®
Monoclonal Antibodies

one antibody, multiple applications™

Cell Signaling
TECHNOLOGY®

XP® Monoclonal Antibodies

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 the most relevant research applications.

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

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 research applications.

# one antibody, multiple

PI3K/Akt Signaling
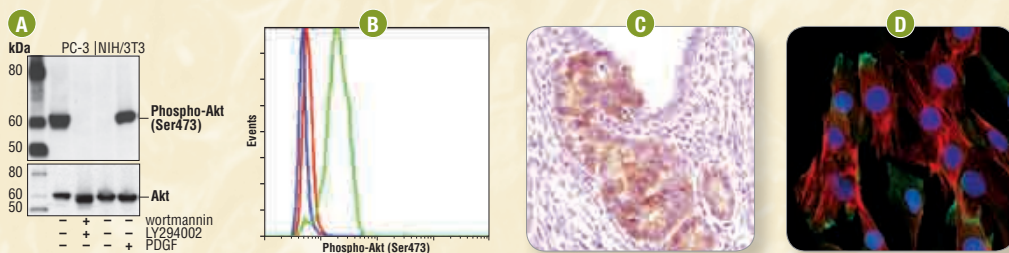
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060: Western blot analysis (**A**) of extracts from PC-3 cells, untreated or treated with LY294002 #9901 and wortmannin #9951, and NIH/3T3 cells, serum-starved or PDGF-treated, using #4060 (upper) or an Akt total protein antibody (lower). Flow cytometric analysis (**B**) of Jurkat cells, untreated (green) or treated with LY294002 #9901, wortmannin #9951, and U0126 #9903 (blue), using #4060 compared to a nonspecific negative control antibody (red). IHC analysis (**C**) of paraffin-embedded PTEN heterozygous mutant mouse endometrium using #4060. (*Tissue section courtesy of Dr. Sabina Signoretti, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*) Confocal IF analysis (**D**) of C2C12 cells treated with insulin, using #4060 (green). Actin filaments were labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

To demonstrate the eXceptional Performance™ of XP® monoclonal antibodies, we compared a number of competitor products side by side in some of the most relevant research applications for these antibodies. Overall, we were able to verify that XP monoclonal antibodies show superior specificity and sensitivity in all tested applications. Moreover, they are also recommended in the broadest range of applications, allowing the use of a single antibody throughout all phases of your research project.

For more information and the most up-to-date list of XP® monoclonal antibodies visit www.cellsignal.com.

applications™

MAP Kinase Signaling
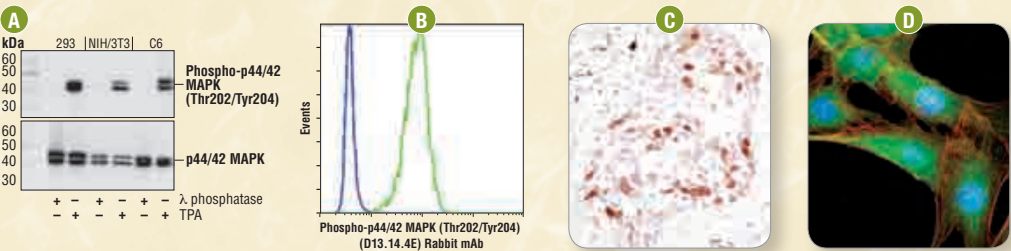
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370: Western blot analysis (**A**) of extracts from 293, NIH/3T3 and C6 cells, treated with λ phosphatase or TPA #4174 as indicated, using #4370 (upper), or a p44/42 MAPK total protein antibody (lower). Flow cytometric analysis (**B**) of Jurkat cells treated with U0126 #9903 (blue) or TPA #4174 (green), using #4370. IHC analysis (**C**) of paraffin-embedded human breast carcinoma using #4370. Confocal IF analysis (**D**) of C2C12 cells, treated with TPA #4174 (200 nM, 15 min), using #4370 (green). Actin filaments were labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).
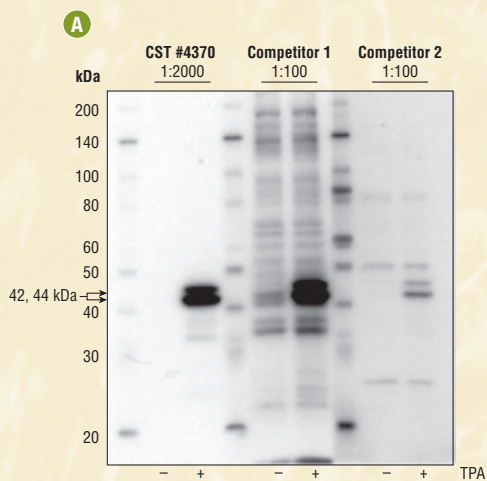
eXceptional Specificity

Antibodies directed against phosphorylated proteins often cross-react with related proteins, resulting in non-specific bands or a false positive signal. We compared Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370 to competing products in a number of relevant research applications.

Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370

- Western blot analysis (**A**) demonstrates the most specific signal with the least amount of background stain for #4370. Notably, the antibody concentration employed is significantly lower for #4370, also attesting to the sensitivity of the product.

- Side by side comparison of #4370 with competitors 1 and 2 in cell-based immunofluorescent (**B**) and immunohistochemical assays (**C**) demonstrates the superior specificity and sensitivity of #4370.

- Western blot analysis (**D**) using a panel of recombinant tyrosine-phosphorylated proteins further confirms the specificity of #4370, but not the competitor products.

- #4370 has the broadest application profile and the greatest number of recommended species, which means only one antibody is needed for detection of phospho-p44/42 MAPK in multiple research applications.

	CST #4370	Competitor 1	Competitor 2
Western Blot Dilution	1:2000	1:100	1:100
Assay Concentration (µg/mL)	0.222	1	10
Recommended Applications	W, IP, IHC-P, IF-IC, F	W, IP, IF	W, IHC-P, IHC-F
Species Cross-reactivity	H, M, R, Hm, Mk, Mi, Dm, Z, B, Dg, Pg, Sc, (Ce)	H, M, R, Dg	H, M, R, (C, X, Z)

A

CST #4370 1:2000 Competitor 1 1:100 Competitor 2 1:100

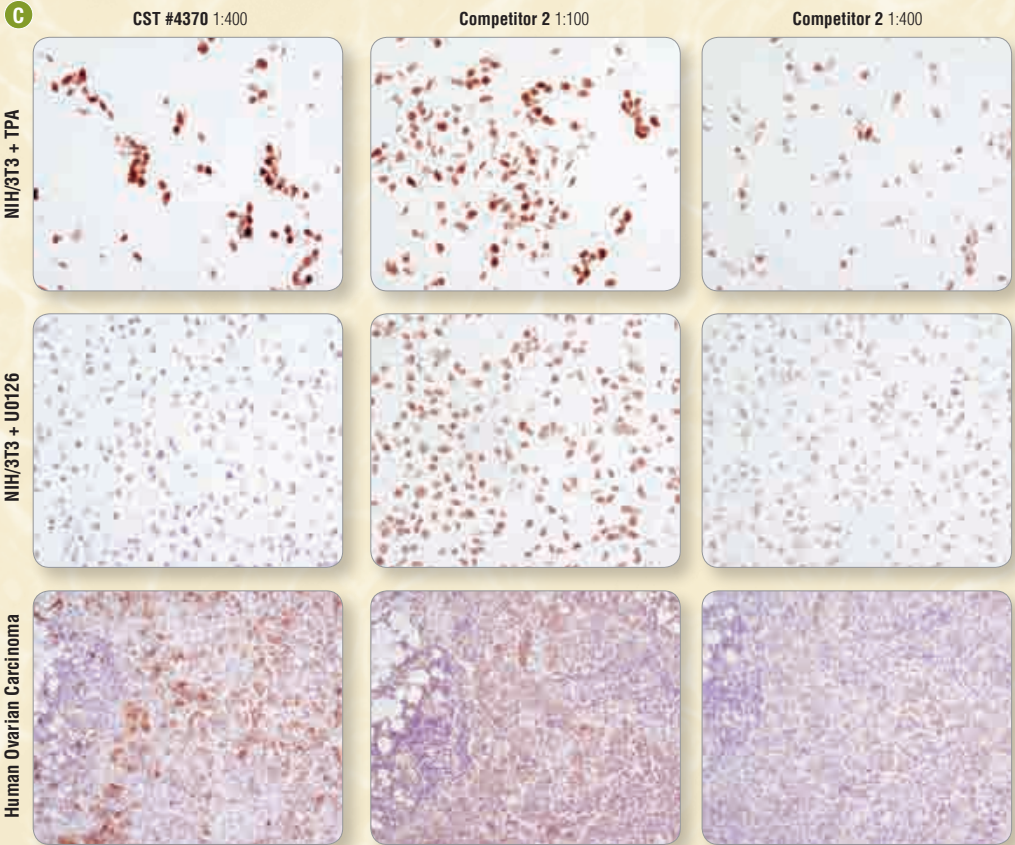kDa: 200, 140, 100, 80, 60, 50, 42, 44 kDa, 40, 30, 20

− + − + − + TPA

Jurkat cells were either left untreated or treated with the phorbol ester TPA #4174 (200 nM,10 min), following overnight serum-deprivation, to induce phosphorylation of p44/42 protein. #4370 was used at the recommended dilution and both competitor antibodies were used at the highest concentration of the manufacturer's recommended dilution range. All antibodies showed the expected signal induction upon TPA treatment. Both competitor antibodies showed greater background staining, with competitor 1 showing a number of cross-reacting bands and competitor 2 also showing weaker staining overall.

B

CST #4370 (1:200)   Competitor 1 (1:50)

HeLa + TPA

HeLa + U0126
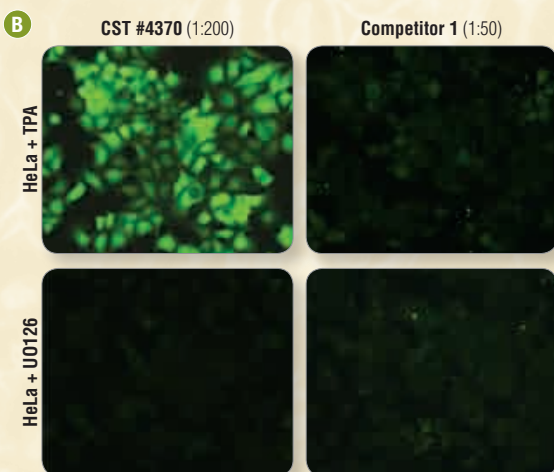
HeLa cells were treated with the MEK 1/2 inhibitor U0126 #9903 (10 µM, 2 hr) or treated with TPA (200 nM, 30 min), following overnight serum deprivation. #4370 was used at the optimal IF-IC recommended dilution of 1:200 and competitor 1 antibody was tested at a dilution range of 1:50-1:500 per the manufacturer's suggestion (data not shown). At the concentration determined optimal, the competitor antibody exhibited more background staining, both nuclear and cytoplasmic, in the U0126-treated cells and only weak, diffuse cytoplasmic staining in the stimulated cells. The expected fold-induction following TPA treatment was observed with #4370, but very little induction was observed in the competitor-stained cells and the antibody appeared "dirtier" overall.

C

| | CST #4370 1:400 | Competitor 2 1:100 | Competitor 2 1:400 |

NIH/3T3 + TPA

NIH/3T3 + U0126

Human Ovarian Carcinoma

#4370 was compared to a competitor's IHC-approved antibody tested at two concentrations. IHC analysis was performed on #8103 [paraffin-embedded NIH/3T3 cell pellets, treated with either TPA #4174 (upper) or U0126 #9903 (middle)] and paraffin-embedded human ovarian carcinoma (lower). At the optimal recommended dilution of 1:400, #4370 showed the appropriate staining in the cell pellet system. The competitor 2 antibody required a 1:400 dilution to eliminate staining in the negative control U0126-treated cells. However, signal in the positive control TPA-treated cells was considerably reduced at this dilution. At 1:100 (lowest suggested dilution), the competitor 2 antibody staining was weak in human ovarian carcinoma tissue and cannot be considered specific given the staining observed in U0126-treated cells. Tissue staining was barely present at a 1:400 dilution of the competitor 2 antibody. In contrast, #4370 demonstrated strong specific staining in human ovarian carcinoma.

D

CST #4370

Competitor 1

Competitor 2

CST Phospho-Tyrosine #9411
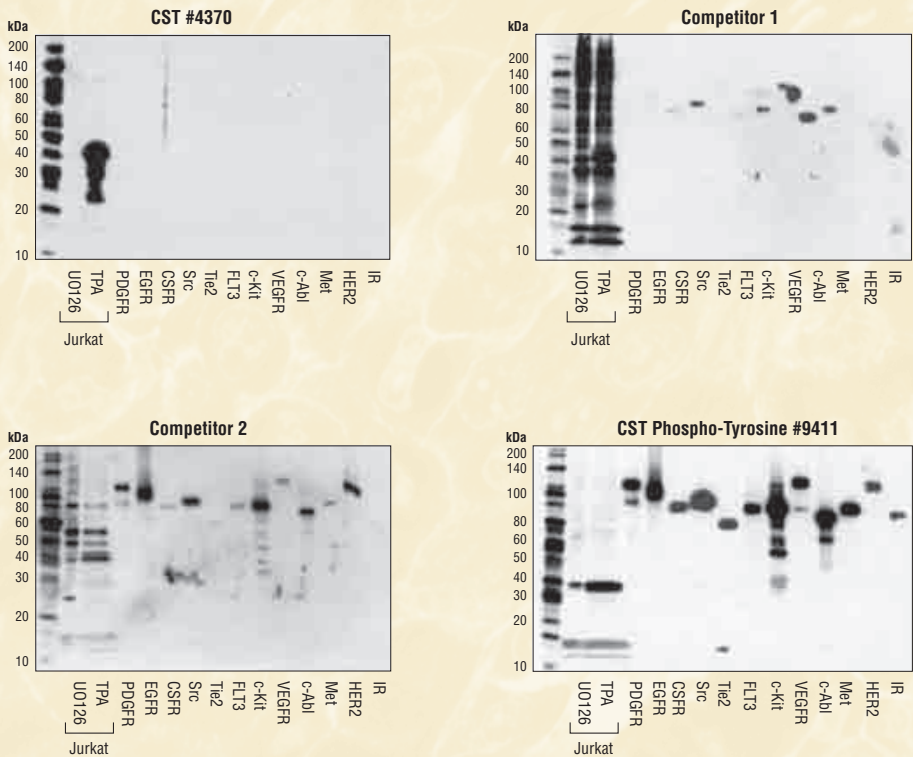
Western blot analysis using a panel of recombinant tyrosine-phosphorylated proteins shows no detectable cross-reactivity using #4370, and significant cross-reactivity with other tyrosine phosphorylated proteins using both competitor antibodies tested. Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 was used to demonstrate protein loading and verify molecular weight of the tagged recombinant proteins. These results demonstrate that #4370 displays exceptional specificity.

eXceptional Sensitivity

While specificity is the most important criterion for a great antibody, sensitivity can become important when research samples are limited or a protein is expressed at low endogenous levels. We compared Phospho-GSK-3β (Ser9) (D85E12) XP® Rabbit mAb #5558 and Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb #4858 with competitor products using western blot analysis.

Phospho-GSK-3β (Ser9) (D85E12) XP® Rabbit mAb #5558

:: Western blot analysis demonstrates the superior specificity and sensitivity of #5558.

:: The concentration of the antibody employed is significantly lower for #5558, further illustrating the sensitivity of the product.

|	CST #5558	Competitor 1	Competitor 2
Western Blot Dilution	1:1000	1:200	1:500, 1:250
Assay Concentration (µg/mL)	0.29	0.5	1
Recommended Applications	W, IP, IF-IC, F	W, IP	W, IF-IC, F
Species Cross-reactivity	H, M, R, Hm	H, M	H
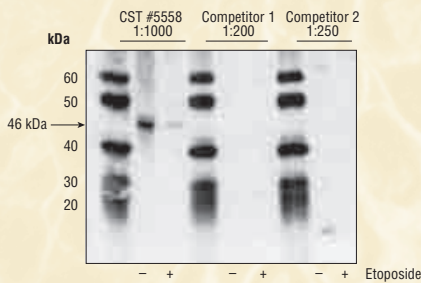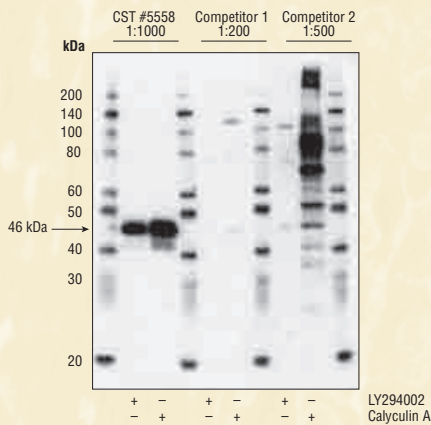
Jurkat cells (**left**) were either treated with the PI3 Kinase inhibitor LY294002 #9901, which inhibits GSK-3β phosphorylation, or with calyculin A #9902 which artificially increases phosphorylation levels by inhibiting phosphatases present in cell extracts. For all antibodies, a signal at the appropriate molecular weight (46 kDa) was detected in the calyculin A-treated sample, however, the signal was strongest with #5558. Note: Competitor 2 also shows a significant number of non-specific bands with greater intensity than the appropriate 46 kDa band. Jurkat cells (**right**) were either left untreated or treated with the apoptosis-inducing reagent, etoposide, a treatment which is known to reduce phosphorylation of GSK-3β. Neither of the competitor products detect phospho-GSK-3β in either the untreated or the etoposide-treated samples.

Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb #4858

:: Western blot analysis demonstrates that #4858 detects protein at lower concentrations than the competing product.

:: The concentration of the antibody employed is significantly lower for #4858, indicating the purity of the product.

:: Due to the high level of specificity and sensitivity of #4858, this antibody displays exceptional performance in a broad range of research applications.

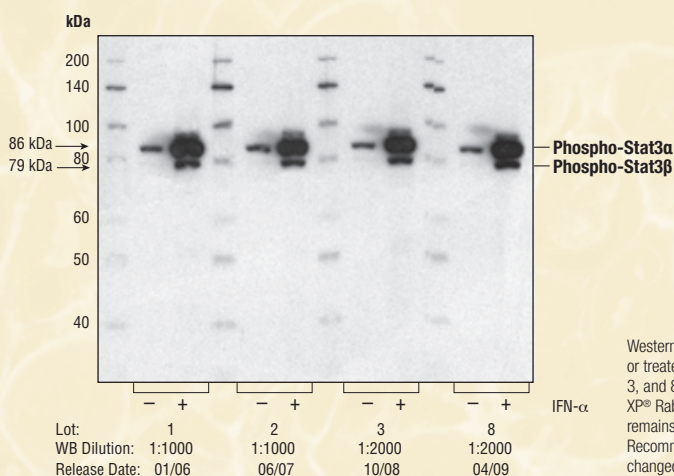|	CST #4858	Competitor 1
Western Blot Dilution	1:2000	1:1000
Assay Concentration (µg/mL)	0.029	Unknown
Recommended Applications	W, IHC-P, IHC-F, IF-IC, F	W
Species Cross-reactivity	H, M, R, Mk, Sc, (C)	H, (R)
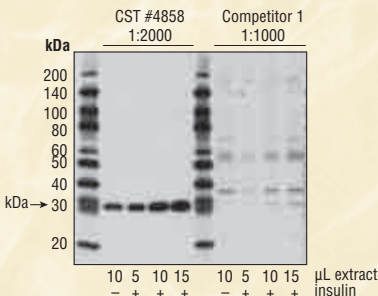
Serial dilutions of extracts from untreated or insulin-treated NIH/3T3 cells (100 nM, 10 min) were detected with #4858 at 1:2000 dilution and the lowest dilution within the manufacturer's recommended range for the competitor antibody (1:1000). The stronger signal was observed using #4858, with the signal of the competitor antibody barely visible in the lanes with lowest amounts of extract or the untreated lane (which contains basal levels of phospho-S6). Note: Cross-reacting bands are stronger using the competitor antibody than the band of appropriate molecular weight showing this product also lacks specificity.
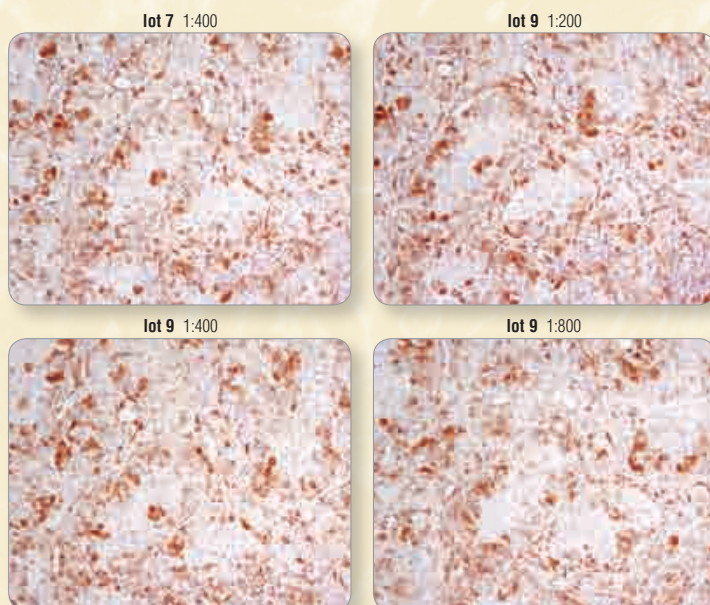
eXceptional Reproducibility

Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145 and Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370 exemplify consistent performance of XP® monoclonal antibodies from lot to lot. Each new lot is always compared to the previous lot in all recommended applications before it becomes available to our customers to ensure the greatest possible consistency in their research.

Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145

Western blot analysis of HeLa cells, untreated or treated with IFN-α comparing lots 1, 2, 3, and 8 of Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145, showing that signal remains consistent from lot to lot. Note: Recommended dilution for western blot was changed to 1:2000 with release of lot 3.

Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370

IHC analysis of adjacent sections of paraffin-embedded human colon carcinoma using #4370 lot 7 at a 1:400 dilution (upper left), #4370 lot 9 at a 1:200 dilution (upper right), #4370 lot 9 at a 1:400 dilution (lower left) and #4370 lot 9 at a 1:800 dilution (lower right). The recommended dilution for lot 9 remained 1:400.

Performance

eXceptional Performance™

Proprietary antibody development technologies, along with our extensive validation and stringent quality control, deliver XP® monoclonal antibodies with exceptional performance in the widest range of research applications.

MAP Kinase Signaling
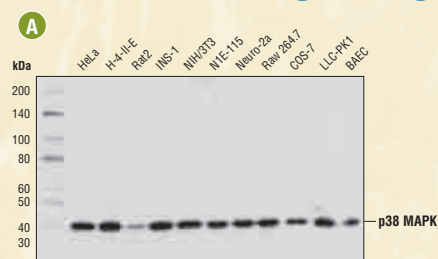
p38 MAPK (D13E1) XP® Rabbit mAb #8690: Western blot analysis (**A**) of extracts from various cell lines using #8690. Flow cytometric analysis (**B**) of HeLa cells using #8690 (blue) compared to concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (red). IHC analysis (**C**) of paraffin-embedded human breast carcinoma using #8690. Confocal IF analysis (**D**) of HeLa cells, untreated (upper) or treated with UV (100 mJ/cm², 30 min recovery; lower), using #8690 (green). Actin filaments were labeled with DY-554 phalloidin (red).
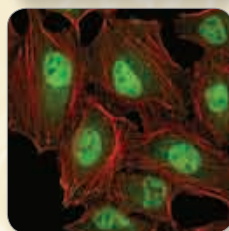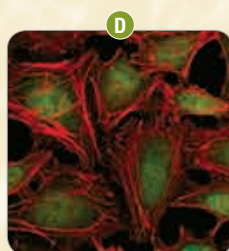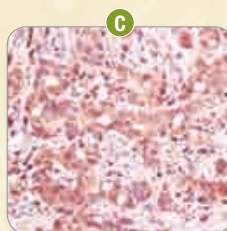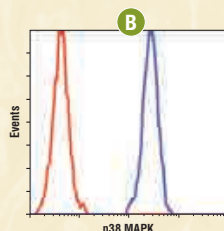
Metabolism
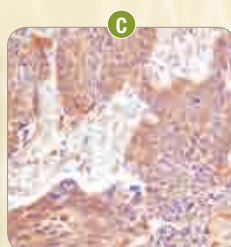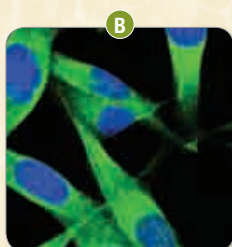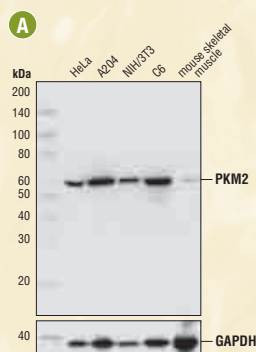
PKM2 (D78A4) XP® Rabbit mAb #4053: Western blot analysis (**A**) of extracts from various cell lines and mouse skeletal muscle using #4053. A GAPDH total protein antibody was used to demonstrate protein loading. Confocal IF analysis (**B**) of A204 cells using #4053 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). IHC analysis (**C**) of paraffin-embedded human lung carcinoma using #4053. IHC analysis (**D**) of paraffin-embedded human lymphoma using #4053.

Neuroscience
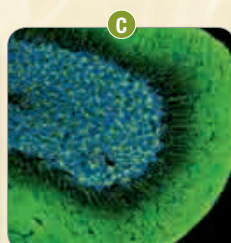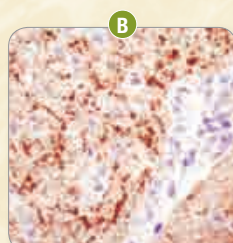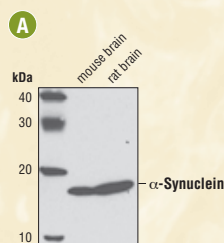
α-Synuclein (D37A6) XP® Rabbit mAb #4179: Western blot analysis (**A**) of extracts from mouse and rat brain using #4179. IHC analysis (**B**) of paraffin-embedded mouse brain using #4179. Confocal IF analysis (**C**) of normal rat cerebellum using #4179 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Autophagy

LC3B (D11) XP® Rabbit mAb #3868: Western blot analysis (**A**) of extracts from various cell lines, untreated (-) or treated overnight with chloroquine (50 μM) (+), using #3868. Flow cytometric analysis (**B**) of HeLa cells using #3868 (blue) compared to a nonspecific negative control antibody (red). IHC analysis (**C**) of paraffin-embedded human astrocytoma using #3868 in the presence of control peptide. Confocal IF analysis (**D**) of HeLa cells, untreated (upper) or chloroquine-treated (lower), using #3868 (green). Actin filaments were labeled using DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).
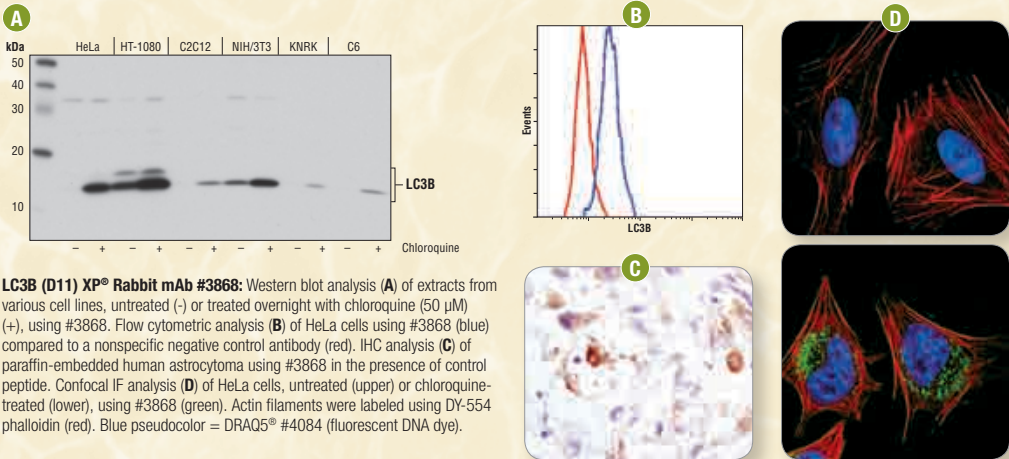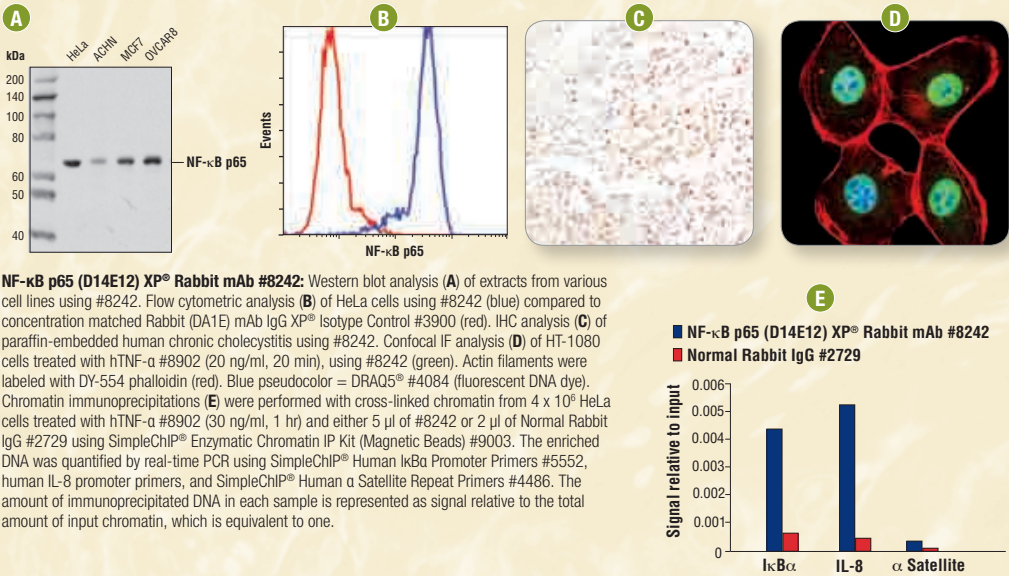
NF-κB Signaling

NF-κB p65 (D14E12) XP® Rabbit mAb #8242: Western blot analysis (**A**) of extracts from various cell lines using #8242. Flow cytometric analysis (**B**) of HeLa cells using #8242 (blue) compared to concentration matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (red). IHC analysis (**C**) of paraffin-embedded human chronic cholecystitis using #8242. Confocal IF analysis (**D**) of HT-1080 cells treated with hTNF-α #8902 (20 ng/ml, 20 min), using #8242 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). Chromatin immunoprecipitations (**E**) were performed with cross-linked chromatin from 4 x 10^6 HeLa cells treated with hTNF-α #8902 (30 ng/ml, 1 hr) and either 5 μl of #8242 or 2 μl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human IκBα Promoter Primers #5552, human IL-8 promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Jak/Stat Signaling
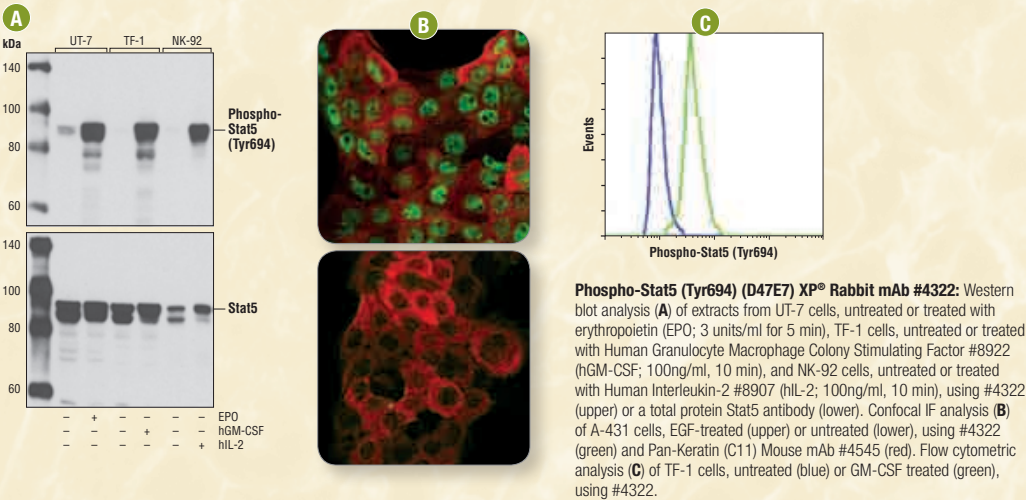
Phospho-Stat5 (Tyr694) (D47E7) XP® Rabbit mAb #4322: Western blot analysis (**A**) of extracts from UT-7 cells, untreated or treated with erythropoietin (EPO; 3 units/ml for 5 min), TF-1 cells, untreated or treated with Human Granulocyte Macrophage Colony Stimulating Factor #8922 (hGM-CSF; 100ng/ml, 10 min), and NK-92 cells, untreated or treated with Human Interleukin-2 #8907 (hIL-2; 100ng/ml, 10 min), using #4322 (upper) or a total protein Stat5 antibody (lower). Confocal IF analysis (**B**) of A-431 cells, EGF-treated (upper) or untreated (lower), using #4322 (green) and Pan-Keratin (C11) Mouse mAb #4545 (red). Flow cytometric analysis (**C**) of TF-1 cells, untreated (blue) or GM-CSF treated (green), using #4322.

Epigenetic Regulation

Ezh2 (D2C9) XP® Rabbit mAb #5246: Western blot analysis (**A**) of extracts from MCF7, Neuro-2a, and COS-7 cell lines using #5246. IHC analysis (**B**) of paraffin-embedded human lymphoma using #5246. IHC analysis (**C**) of paraffin-embedded human cervical carcinoma using #5246. Confocal IF analysis (**D**) of HeLa cells using #5246 (green) and S6 Ribosomal Protein (54D2) Mouse mAb #2317 (blue). Actin filaments were labeled with DY-554 phalloidin (red). Chromatin immunoprecipitations (**E**) were performed with cross-linked chromatin from 4×10^6 NCCIT cells and either 5 µl of #5246 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human HoxA1 Intron 1 Primers #7707, SimpleChIP® Human HoxA2 Promoter Primers #5517, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.
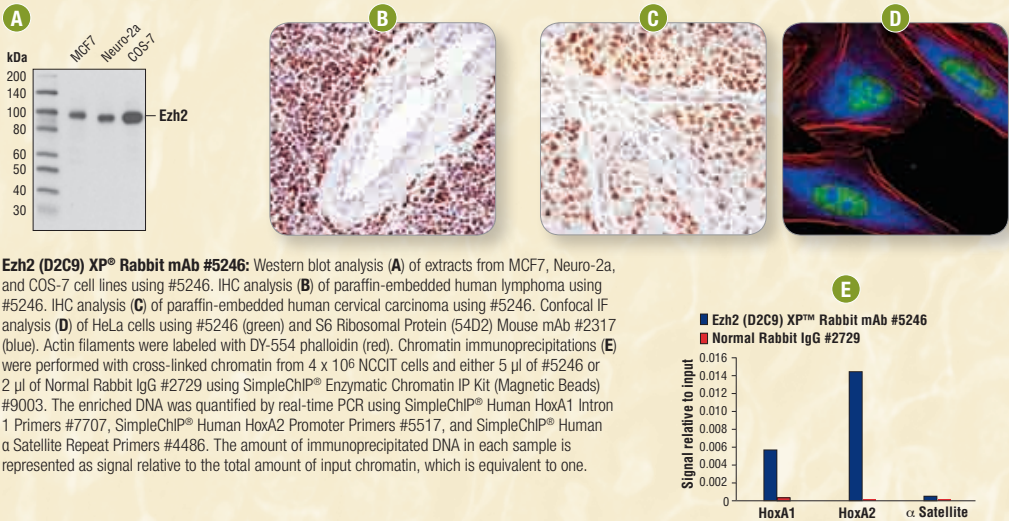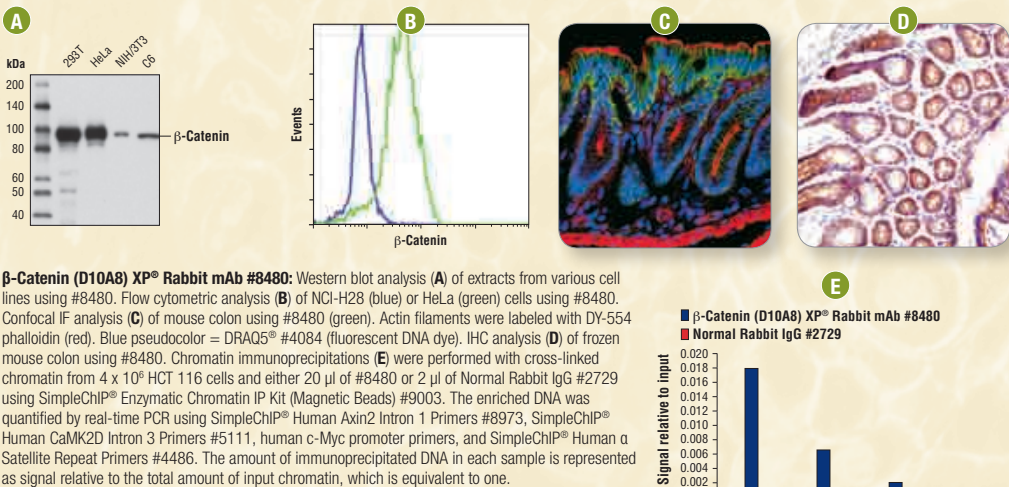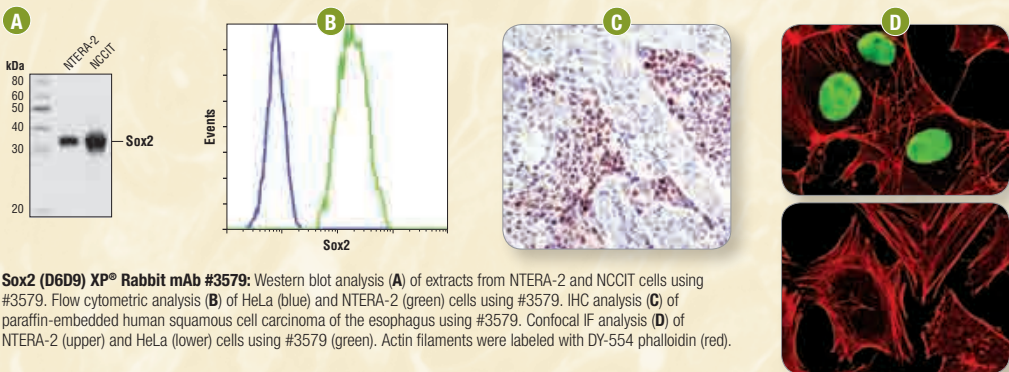
Development and Differentiation

β-Catenin (D10A8) XP® Rabbit mAb #8480: Western blot analysis (**A**) of extracts from various cell lines using #8480. Flow cytometric analysis (**B**) of NCI-H28 (blue) or HeLa (green) cells using #8480. Confocal IF analysis (**C**) of mouse colon using #8480 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). IHC analysis (**D**) of frozen mouse colon using #8480. Chromatin immunoprecipitations (**E**) were performed with cross-linked chromatin from 4×10^6 HCT 116 cells and either 20 µl of #8480 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human Axin2 Intron 1 Primers #8973, SimpleChIP® Human CaMK2D Intron 3 Primers #5111, human c-Myc promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Stem Cell Markers

Sox2 (D6D9) XP® Rabbit mAb #3579: Western blot analysis (**A**) of extracts from NTERA-2 and NCCIT cells using #3579. Flow cytometric analysis (**B**) of HeLa (blue) and NTERA-2 (green) cells using #3579. IHC analysis (**C**) of paraffin-embedded human squamous cell carcinoma of the esophagus using #3579. Confocal IF analysis (**D**) of NTERA-2 (upper) and HeLa (lower) cells using #3579 (green). Actin filaments were labeled with DY-554 phalloidin (red).

PI3K/Akt Signaling

Phospho-NDRG1 (Thr346) (D98G11) XP® Rabbit mAb #5482: Western blot analysis (**A**) of HeLa cells treated with hEGF #8916 (100 ng/ml, 30 minutes), mock transfected or transfected with SignalSilence® NDRG1 siRNA I #6245 or SignalSilence® NDRG1 siRNA II #6257, using #5482 (upper) or a β-Tubulin total protein antibody (lower). Flow cytometric analysis (**B**) of Jurkat cells, untreated (green) or treated with LY294002 #9901, wortmannin #9951 and U0126 #9903 (blue), using #5482. IHC analysis (**C**) of paraffin-emebedded human lung carcinoma using #5482. Confocal IF analysis (**D**) of C2C12 cells, treated with LY294002 #9901 (upper) or insulin (lower), using #5482 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

XP®

one antibody, multiple applications™

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