



Cell Signaling

TECHNOLOGY®

ANNOUNCING...

XMT™

eXceptional

Monoclonal

Technology™

XP™ Monoclonal Antibodies



eXceptional Monoclonal Technology™

XMT™, an eXceptional Monoclonal Technology™ from Cell Signaling Technology®, enables the development and production of antibodies with eXceptional Performance™ (XP™). This revolutionary and proprietary technology provides us with 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 of our 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.

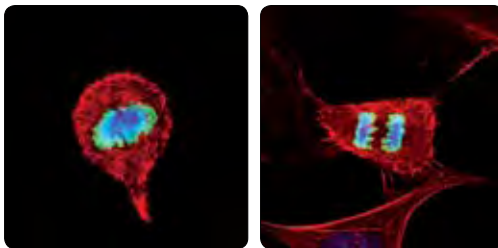
+ eXceptional stability and reproducibility

XMT combined with our stringent quality control ensures maximum lot-to-lot consistency and the most reproducible results.

= eXceptional performance

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

#3377 XP™ Rabbit mAb



Confocal immunofluorescent analysis of HeLa cells using **Phospho-Histone H3 (Ser10) (D2C8) XP™ Rabbit mAb #3377** (green). Actin filaments have been labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

eXceptional Specificity

A common problem with commercially available antibodies directed against phosphorylated receptor tyrosine kinases (RTKs) is crossreactivity with related active RTKs, resulting in a false positive signal. Comparison of **Phospho-Met (Tyr1234/1235) (D26) XP™ Rabbit mAb #3077** with a competitor's product illustrates the value of high performance XMT-derived products from Cell Signaling Technology combined with our extensive product validation.

- Side by side comparison of #3077 and a competitor's product gives the appearance of specific immunohistochemical staining for both products (Figure 1).
- Western blot analysis confirms a single and specific band of the appropriate molecular weight for #3077, but not for the competitor's antibody (Figure 2).
- Paraffin-embedded cell pellets treated with various RTK inhibitors and stimulators confirm specificity by IHC of #3077 but not of the competitor's antibody (Figure 3).
- Specificity, sensitivity and performance of #3077 was further confirmed by IHC using tissue samples and cell pellets (visit www.cellsignal.com/xmt for further details).

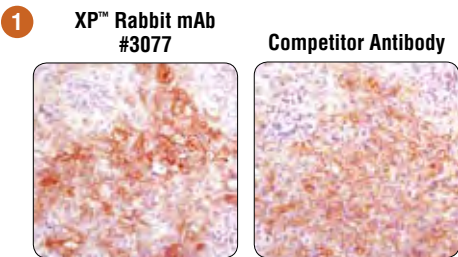


Fig. 1. IHC analysis of paraffin-embedded HCC827 xenograft comparing **Phospho-Met (Tyr1234/1235) (D26) XP™ Rabbit mAb #3077** (left) and a competitor's product (right) gives the appearance of specific staining for both products.

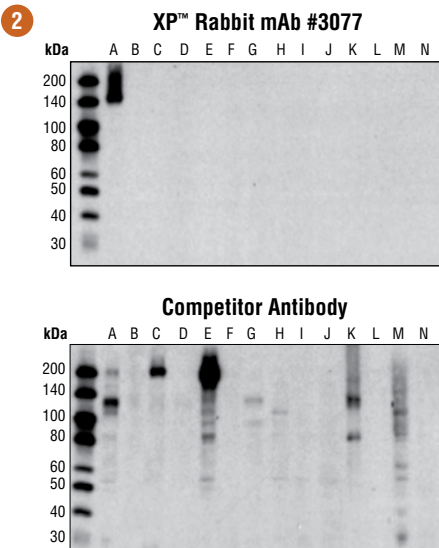


Fig. 2. A single band at 145 kDa was observed by western blotting in HGF-stimulated, but not in unstimulated A431 cells using **Phospho-Met (Tyr1234/1235) (D26) XP™ Rabbit mAb #3077**. Extracts treated with growth factors that activate other RTKs or that overexpress other RTKs or cytoplasmic tyrosine kinases were negative (top). By comparison, the competitor phospho-Met antibody recognizes several nonspecific bands (bottom). Both membranes were developed on the same film with the same exposure time (10 seconds).

A.	A431 + HGF
B.	A431 - HGF
C.	NIH/3T3 + PDGF
D.	NIH/3T3 - PDGF
E.	A431 + EGF
F.	COS/FGFR1
G.	CHO/IRS1,IR + Insulin
H.	K562
I.	SUPM2
J.	L540
K.	NIH/3T3/src
L.	GST-Tie-2
M.	SEM
N.	H526+SCF

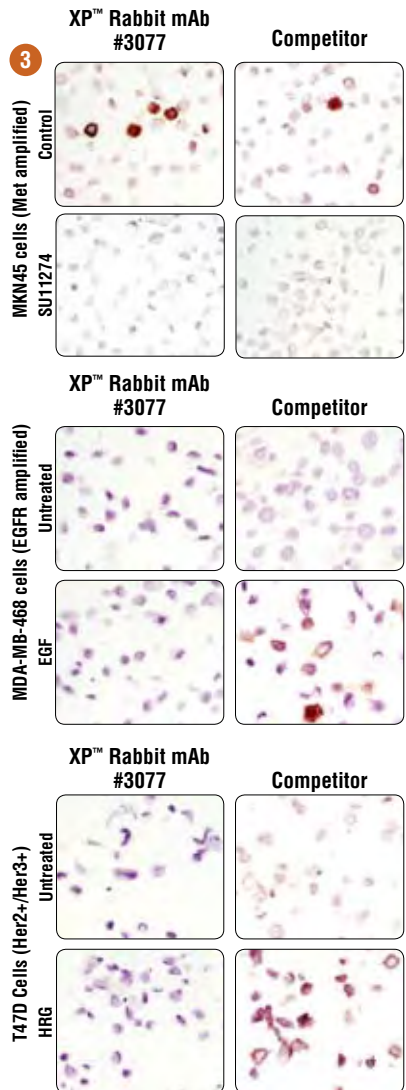


Fig. 3. IHC analysis of paraffin-embedded MKN45 cells treated with the Met inhibitor SU11274 showed no staining as compared to the control using both **Phospho-Met (Tyr1234/1235) (D26) XP™ Rabbit mAb #3077** and the competitor's antibody. EGF treatment and HRG treatment of MDA-MB-468 and T47D breast carcinoma cell lines, respectively, showed no increase in staining compared to control using #3077. In contrast, the competitor's antibody detected non-specific staining with both treatments, indicating cross-reactivity with other RTKs.

eXceptional Sensitivity

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

exemplifies the exceptional sensitivity of XP monoclonal antibodies from Cell Signaling Technology (CST). We compared XMT-derived #4858 to #4856, a rabbit monoclonal antibody generated using conventional monoclonal technologies. As shown in Figure 1A and B, both CST antibodies show a single and specific band of the appropriate molecular weight, consistent with our high standard validation requirements and promise of the highest quality.

- XMT-derived #4858 detects target-specific signal at lower concentrations compared to #4856, indicating #4858 has superior sensitivity and affinity (Figure 1A).
- Selling stocks of #4858 detect lower lysate concentrations than #4856 (Figure 1B).
- Quantification of immunofluorescence intensity in a serial dilution revealed greater intensity of #4858 compared to #4856 (Figure 2A).
- Using confocal immunofluorescent analysis at matched concentrations a slightly brighter signal is seen with #4858 (Figure 2B).
- Side by side comparisons using immunohistochemistry and flow cytometry also confirmed superior sensitivity of #4858 (for more details visit www.cellsignal.com/xmt).

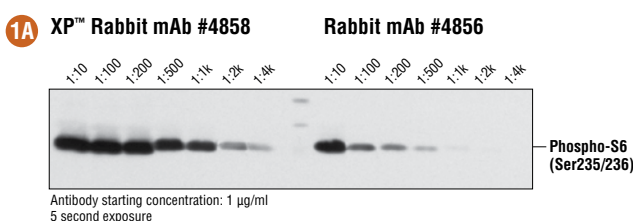


Fig. 1A. Western blot analysis of extracts from insulin-treated HeLa cells using serial antibody dilutions of **Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb #4858** and **Phospho-S6 Ribosomal Protein (Ser235/236) (2F9) Rabbit mAb #4856**, starting at 1 µg/ml.

Both antibodies generate a clean and specific signal. However, a much more intense signal was observed with #4858. On the 5 second exposure shown, #4858 generates a strong signal at 1 ng/ml, while the signal from #4856 is much weaker.

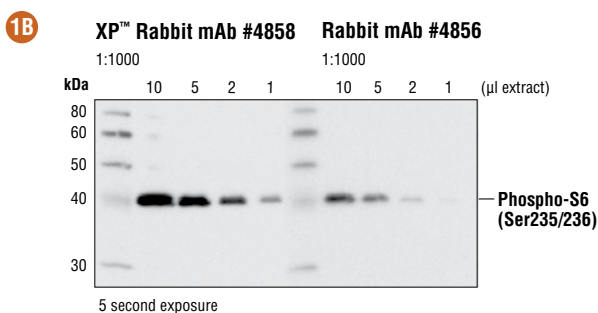


Fig. 1B. Western blot analysis of a serial dilution of extracts from insulin-treated HeLa cells (100 nM for 10 min.) detected with our selling stocks of #4858 and #4856 at a 1:1000 dilution. Again, the strongest signal was observed using #4858.

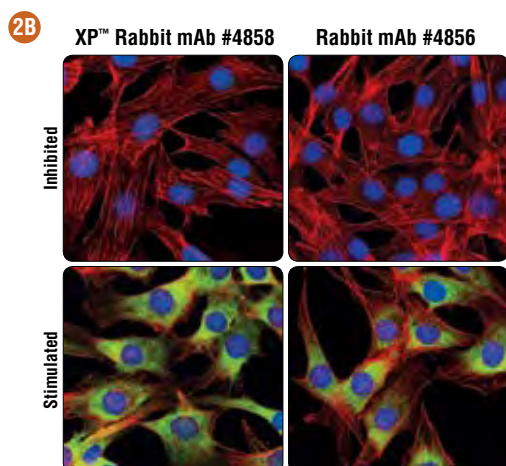
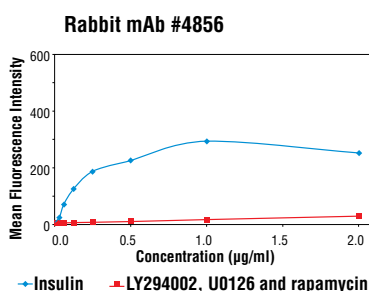
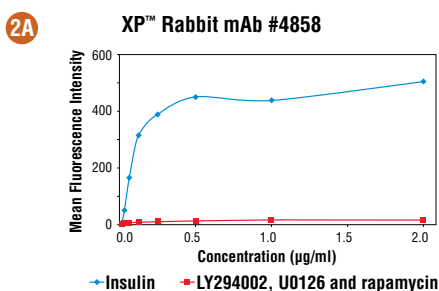


Fig. 2A. Quantitative IF titration analysis comparing **Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb #4858** to **Phospho-S6 Ribosomal Protein (Ser235/236) (2F9) Rabbit mAb #4856** on C2C12 cells inhibited with a combination of LY294002, U0126, and rapamycin for 2 hours or stimulated with insulin for 30 minutes. A comparison of the two treatment conditions revealed an optimal working concentration of 0.25 µg/ml for both antibodies. However, at this concentration, #4858 signal intensity was 2-fold higher than #4856, demonstrating greater sensitivity. Data were generated using an Acumen® high content screening platform.

Fig. 2B. Confocal IF analysis of inhibited (upper) or stimulated (lower) C2C12 cells using #4858 (left, green) or #4856 (right, green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). At their optimal working concentrations, #4858 produces a stronger signal than #4856.

eXceptional Reproducibility

Phospho-Stat3 (Tyr705) (D3A7) XP™ Rabbit mAb #9145 and **Jak2 (D2E12) XP™ Rabbit mAb #3230** exemplify the lot-to-lot consistency achieved using XMT coupled with the stringent quality control standards applied to all antibodies from Cell Signaling Technology.

XP™ Rabbit mAb #9145

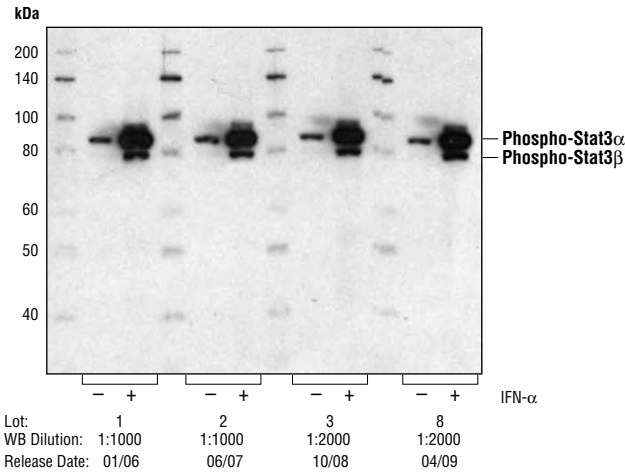


Fig. 1. Western blot analysis of HeLa cells, untreated or treated with IFN- α comparing lot 1, 2, 3 and 8 of **Phospho-Stat3 (Tyr705) (D3A7) XP™ Rabbit mAb #9145**, showing that signal remains consistent from lot to lot. Note: Recommended dilution for western blot was changed to 1:2000 with release of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

XP™ Rabbit mAb #3230

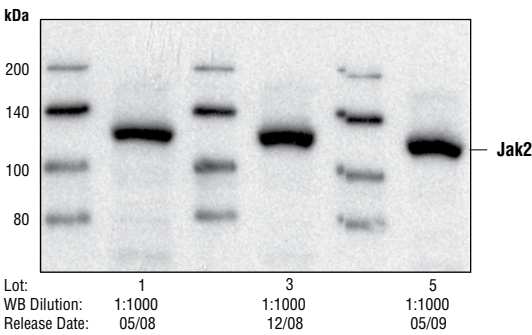
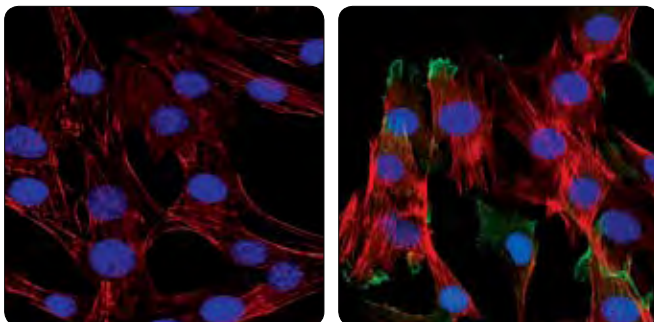


Fig. 2. Western blot analysis of K562 cells comparing lot 1, 3 and 5 of **Jak2 (D2E12) XP™ Rabbit mAb #3230**, showing that signal remains consistent from lot to lot.

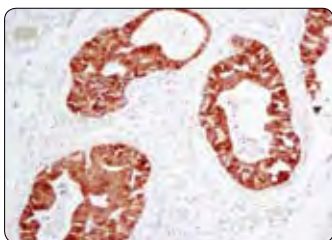
With XP™ monoclonal antibodies we provide you with our highest performing antibody without an increased price, for a greater overall value.

eXceptional Performance™

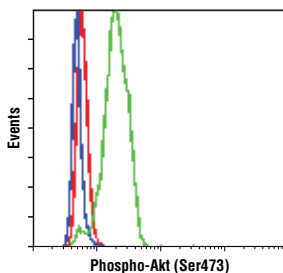
Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060 is an example of an antibody with superior performance in all tested applications, previously unattainable with traditional approaches (see Table below).



Confocal immunofluorescent analysis of C2C12 cells, LY294002-treated (left) or insulin-treated (right), using **Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060** (green). Actin filaments have been labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Immunohistochemical analysis of paraffin-embedded PTEN (-/-) mouse prostate using **Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060**. Tissue courtesy of Dr. David Guertin, The Whitehead Institute of Biomedical Research, Cambridge, MA.



Flow cytometric analysis of Jurkat cells, untreated (green) or treated with LY294002, wortmannin and U0126 (blue), using **Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060** compared to a nonspecific negative control antibody (red).

No.	Antibody	Reactivity	WB	IP	IHC	Flow	IF
4060	Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb	H, M, R, Hm, B, Dm, Z, (Mk, C, Dg)	++++	++++	++++	++++	++++
4058	Phospho-Akt (Ser473) (193H12) Rabbit mAb	H, M, R	+++	+++	-	+++	+++
3787	Phospho-Akt (Ser473) (736E11) Rabbit mAb	H, M, (R)	-	-	++	-	-
9271	Phospho-Akt (Ser473) Antibody	H, M, R, Pg, C, Hm, B, Dm, Dg, (Mk)	+++	++	-	++	++
4051	Phospho-Akt (Ser473) (587F11) Mouse mAb	H, M, R, Hm, (Mk)	+++	+++	-	-	-

Table 1. Summary of Phospho-Akt (Ser473) Antibodies available from Cell Signaling Technology. Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Applications Key: WB=Western Blot / IP=Immunoprecipitation / IHC=Immunohistochemistry / Flow=Flow Cytometry / IF=Immunofluorescence

Testing Data Key: (++++)=Very Highly Recommended / (+++)=Highly Recommended / (++)=Recommended / (-)=Not Recommended

Reactivity Key: H=Human / M=Mouse / R=Rat / Mk=Monkey / Hm=Hamster / B=Bovine / Dm=D. melanogaster / Z=Zebra Fish / C=Chicken / Dg=Dog / Pg=Pig / Species enclosed in parentheses are predicted to react based on 100% sequence homology.

**Find out more at
www.cellsignal.com/xmt**

**With XP™ monoclonal antibodies we provide
you with our highest performing antibody
without an increased price,
for a greater overall value.**

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