Validated Antibodies for Western Blotting

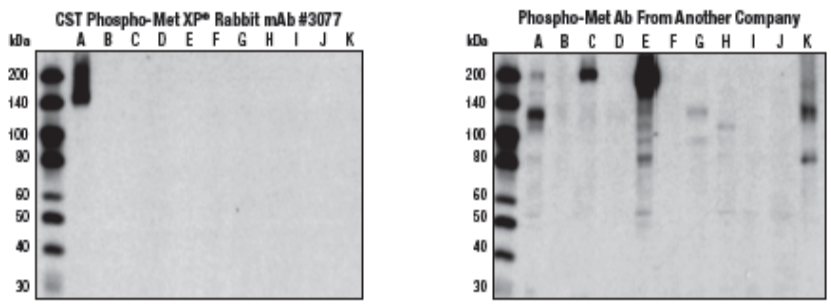
CST™ antibodies are thoroughly tested for specificity, sensitivity, and reproducibility, so you don't have to spend time or use your samples on validation. They are supported by optimized protocols, companion reagents, reference information, and technical support to ensure your success.

Is your antibody specific?

CST has already evaluated each antibody by multiple methods using biologically relevant systems so you can be confident in the specificity of your antibody.

Panel Testing

Specificity testing using a panel of cell lines and treatments demonstrates that #3077 is specific for phospho-Met with no detectable cross-reactivity with other tyrosine kinases. In comparison, a phospho-Met antibody from another company shows significant cross-reactivity.



Tyrosine Kinase Cross-reactivity Panel: Cell Lines & Treatments

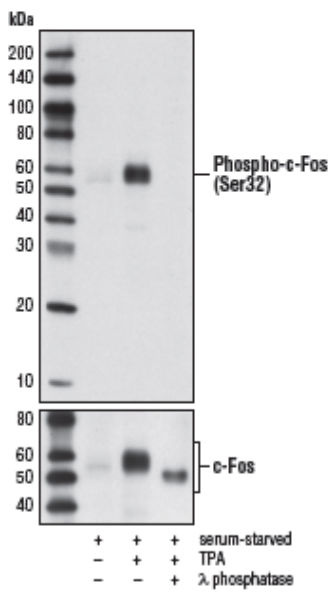
A. A-431 + HGF	**D.** NIH/3T3 (untreated)	**G.** CHO/IRS1,IR + Insulin	**J.** L-540
B. A-431 (untreated)	**E.** A-431 + EGF	**H.** K-562	**K.** NIH/3T3/src
C. NIH/3T3 + PDGF	**F.** COS/FGFR1	**I.** SUP-M2	

Phospho-Met (Tyr1234/1235) (D26) XP® Rabbit mAb #3077: A single band at 145 kDa was observed by WB in HGF-stimulated, but not in unstimulated A-431 cells **(A & B)**, using #3077 (left). Extracts from cells expressing other receptor tyrosine kinases (RTKs) or cytoplasmic tyrosine kinases were negative with the CST antibody.

Activator/Inhibitor Treatment

Treatment of cells with activators or inhibitors, to induce or inhibit target expression, verifies specificity. Phosphatase treatment confirms phospho-specificity.

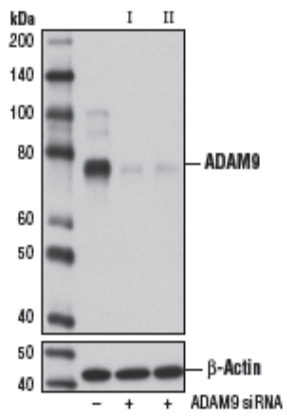
Phospho-c-Fos (Ser32) (D82C12) XP® Rabbit mAb #5348: Western blot analysis of extracts from HeLa cells, serum-starved overnight and then either untreated or stimulated for 4 hr with TPA #4174, using #5348 (upper) and c-Fos (9F6) Rabbit mAb #2250 (lower). Antibody phospho-specificity is shown by treating lysates with λ-phosphatase.



siRNA Knockdown

The use of siRNA transfection verifies target specificity.

SignalSilence® ADAM9 siRNA I #11968: Analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), #11968 (+), or SignalSilence® ADAM9 siRNA II (+), using ADAM9 Rabbit mAb #4151 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The ADAM9 Rabbit mAb #4151 confirms silencing of ADAM9 expression, while the β-Actin (D6A8) Rabbit mAb is used as a loading control.



Analysis of Multiple Cell Lines

Analysis of multiple cell lines and/or tissues of known expression levels allows accurate determination of species cross-reactivity and verification of specificity.

Androgen Receptor (D6F11) XP® Rabbit mAb #5153: Western blot analysis of extracts from LNCaP (AR+), MCF7 (AR+), PC-3 (AR-), and DU 145 (AR-) cells using #5153 (upper) and β-Actin Antibody #4967 (lower).