#8619 Store at -20°C

Phospho-AS160 (Ser318) (D3D11) Rabbit mAb

Cell Signaling TECHNOLOGY®

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info@cellsignal.com
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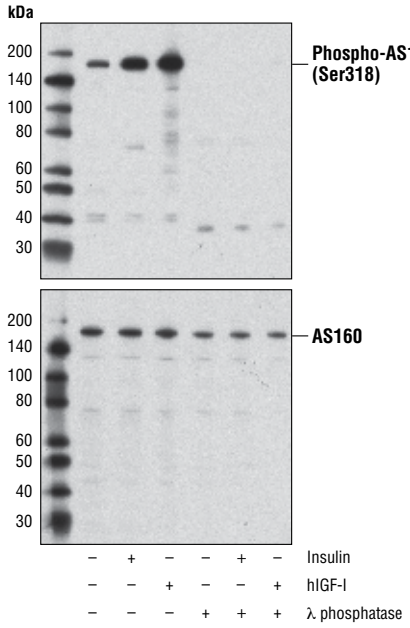
Entrez-Gene ID #9882
Swiss-Prot Acc. #O60343

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP Endogenous	H, (M, R)	160 kDa	Rabbit IgG**

Background: The polypeptide insulin is a major hormone controlling critical energy functions such as glucose and lipid metabolism. Insulin binds to and activates the insulin receptor (IR) tyrosine kinase, which phosphorylates and recruits different adaptor proteins. The signaling pathway initiated by insulin and its receptor stimulates glucose uptake in muscle cells and adipocytes through translocation of GLUT4 glucose transporter from the cytoplasm to the plasma membrane (1). A 160 kDa substrate of the Akt Ser/Thr kinase (AS160, TBC1D4) is a Rab GTPase activating protein that regulates insulin-stimulated GLUT4 trafficking. AS160 is expressed in many tissues including brain, kidney, liver, and brown and white fat (2). Multiple Akt phosphorylation sites were identified on AS160 *in vivo*, with five sites (Ser318, Ser570, Ser588, Thr642, and Thr751) showing increased phosphorylation following insulin treatment (2,3). Studies using recombinant AS160 demonstrated that insulin-stimulated phosphorylation of AS160 is a crucial step in GLUT4 translocation (3) and is reduced in some patients with type 2 diabetes (4). The interaction of 14-3-3 regulatory proteins with AS160 phosphorylated at Thr642 is a necessary step for GLUT4 translocation (5). Phosphorylation of AS160 by AMPK is involved in the regulation of contraction-stimulated GLUT4 translocation (6).

Specificity/Sensitivity: Phospho-AS160 (Ser318) (D3D11) Rabbit mAb recognizes endogenous levels of AS160 protein only when phosphorylated at Ser318.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser318 of human AS160 protein.



Western blot analysis of extracts from serum-starved HeLa cells, untreated or treated with either insulin (150 nM, 15 min) or hIGF-I #8917 (100 ng/ml, 15 min), using Phospho-AS160 (Ser318) (D3D11) Rabbit mAb (upper) or AS160 (C69A7) Rabbit mAb #2670 (lower). The phospho-specificity of the antibody is verified by λ phosphatase treatment.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Watson, R.T. and Pessin, J.E. (2006) *Trends Biochem. Sci.* 31, 215-222.

(2) Kane, S. et al. (2002) *J. Biol. Chem.* 277, 22115-22118.

(3) Sano, H. et al. (2003) *J. Biol. Chem.* 278, 14599-14602.

(4) Karlsson, H.K. et al. (2005) *Diabetes* 54, 1692-1697.

(5) Ramm, G. et al. (2006) *J. Biol. Chem.* 281, 29174-29180.

(6) Kramer, H.F. et al. (2006) *J. Biol. Chem.* 281, 31478-31485.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.