Store at -20°C

#59633

AS160 Signaling Antibody Sampler Kit

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
AS160 (C69A7) Rabbit mAb	2670	20 µl	160 kDa	Rabbit IgG
Phospho-AS160 (Ser318) (D3D11) Rabbit mAb	8619	20 µl	160 kDa	Rabbit IgG
Phospho-AS160 (Ser588) (D8E4) Rabbit mAb	8730	20 µl	160 kDa	Rabbit IgG
Phospho-AS160 (Thr642) (D27E6) Rabbit mAb	8881	20 µl	160 kDa	Rabbit IgG
Akt (pan) (C67E7) Rabbit mAb	4691	20 µl	60 kDa	Rabbit IgG
Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb	13038	20 µl	60 kDa	Rabbit IgG
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	4060	20 µl	60 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The AS160 Signaling Antibody Sampler Kit provides an economical means of detecting select components involved in the AS160 signaling pathway. The kit contains enough primary antibodies to perform at least two western blot experiments per antibody.

Background: 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 adaptor proteins. The signaling pathway initiated by insulin and its receptor stimulates glucose uptake in muscle cells and adipocytes through translocation of the 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 have been 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 demonstrate 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).

Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (7-9). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (8,9). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (10) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as the mammalian target of rapamycin (mTOR) in a rapamycin-insensitive complex with rictor and Sin1 (11,12).

Specificity/Sensitivity: AS160 (C69A7) Rabbit mAb detects endogenous levels of total AS160 protein. Phospho-AS160 (Ser318) (D3D11) Rabbit mAb recognizes endogenous levels of AS160 protein only when phosphorylated at Ser318. Phospho-AS160 (Ser588) (D8E4) Rabbit mAb recognizes endogenous levels of AS160 protein only when phosphorylated at Ser588. Phospho-AS160 (Thr642) (D27E6) Rabbit mAb recognizes endogenous levels of AS160 protein only when phosphorylated at Thr642. Akt (pan) (C67E7) Rabbit mAb detects endogenous levels of total Akt protein. This antibody does not cross-react with other related proteins. Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb recognizes endogenous levels of Akt1 protein only when phosphorylated at Thr308. This antibody also recognizes endogenous levels of Akt2 protein when phosphorylated at Thr309 or Akt3 protein when phosphorylated at Thr305. Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb detects endogenous levels of Akt only when phosphorylated at Ser473.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ala195 of human AS160. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser318 of human AS160 protein. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser588 of human AS160 protein. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr642 of human AS160 protein. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues in the carboxy-terminal sequence of mouse Akt. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Thr308 of human Akt1 protein. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser473 of human Akt.

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.*

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

Background References:

(1) Watson, R.T. and Pessin, J.E. (2006) *Trends Biochem. Sci.* 31, 215-22.
(2) Kane, S. et al. (2002) *J. Biol. Chem.* 277, 22115-8.
(3) Sano, H. et al. (2003) *J. Biol. Chem.* 278, 14599-602.
(4) Karlsson, H.K. et al. (2005) *Diabetes* 54, 1692-7.
(5) Ramm, G. et al. (2006) *J. Biol. Chem.* 281, 29174-80.
(6) Kramer, H.F. et al. (2006) *J. Biol. Chem.* 281, 31478-85.
(7) Franke, T.F. et al. (1997) *Cell* 88, 435-7.
(8) Burgering, B.M. and Coffer, P.J. (1995) *Nature* 376, 599-602.
(9) Franke, T.F. et al. (1995) *Cell* 81, 727-36.
(10) Alessi, D.R. et al. (1996) *EMBO J* 15, 6541-51.
(11) Sarbassov, D.D. et al. (2005) *Science* 307, 1098-101.
(12) Jacinto, E. et al. (2006) *Cell* 127, 125-37.

U.S. Patent No. 7,429,487, foreign equivalents, and child patents deriving therefrom.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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.

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.