#9270 Store at -20°C

PhosphoPlus® Akt (Ser473) Antibody Kit

10 Western Blots

Cell Signaling TECHNOLOGY®

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rev. 08/05/16

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

Entrez-Gene ID #207
UniProt ID #P31749

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	4060	100 µl	60 kDa	Rabbit IgG
Akt (pan) (C67E7) Rabbit mAb	4691	100 µl	60 kDa	Rabbit IgG
Akt Control Cell Extracts	9273	200 µl		
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-biotin, HRP-linked Antibody	7075	100 µl		Goat
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml		
Biotinylated Protein Ladder	7727	100 µl		

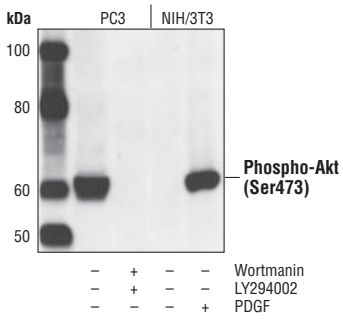
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The PhosphoPlus® Akt (Ser473) Antibody Kit provides reagents and protocols for the rapid analysis of Akt phosphorylation.

Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTor) in a rapamycin-insensitive complex with rictor and Sin1 (5,6). Akt promotes cell survival by inhibiting apoptosis by phosphorylating and inactivating several targets, including Bad (7), forkhead transcription factors (8), c-Raf (9) and caspase-9. PTEN phosphatase is a major negative regulator of the PI3 kinase/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11).

Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12).

In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (15) and p21 Waf1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18). Inhibition of mTOR stops the protein synthesis machinery due to inactivation of its effector, p70 S6 kinase and activation of the eukaryotic initiation factor 4E binding protein 1 (4E-EP1), an inhibitor of translation (18,19).



Western blot analysis of extracts from PC3 cells, untreated or LY294002/wortmannin-treated, and NIH/3T3 cells, serum-starved or PDGF-treated, using ***Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060.***

Specificity/Sensitivity: Phospho-Akt (Ser473) Antibody detects endogenous levels of Akt1 only when phosphorylated at Ser473, and Akt2 and Akt3 when phosphorylated at equivalent sites. It does not recognize Akt phosphorylated at other sites, nor does it recognize phosphorylated forms of related kinases such as PKC or p70 S6 kinase. Akt Antibody detects endogenous levels of total Akt1, Akt2 and Akt3 proteins. It does not cross-react with related kinases.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser473 or the carboxy-terminal sequence of Akt.

Control Proteins: Nonphosphorylated Akt Cell Extracts: Total cell extracts from Jurkat cells, serum starved overnight and then treated with 50 µM LY294002 (CST #9901) for one hour, serve as a negative control. Supplied in SDS Sample Buffer.Phosphorylated Akt Cell Extracts: Total cell extracts from Jurkat cells, serum starved overnight and then treated with Calyculin A (CST #9902) to preserve their activated Akt state, serve as a positive control. Supplied in SDS Sample Buffer..

Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20ºC. Do not aliquot the antibodies.

Akt Control Cell Extracts are supplied in SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25ºC), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red. Store at -20ºC, or at -80ºC for long-term storage.

Recommended Antibody Dilutions:
Western blotting 1:1000

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

Background References:

(1) Franke, T.F. et al. (1997) *Cell* 88, 435–7.
(2) Burgering, B.M. and Coffer, P.J. (1995) *Nature* 376, 599–602.
(3) Franke, T.F. et al. (1995) *Cell* 81, 727–36.
(4) Alessi, D.R. et al. (1996) *EMBO J* 15, 6541–51.
(5) Sarbassov, D.D. et al. (2005) *Science* 307, 1098–101.
(6) Jacinto, E. et al. (2006) *Cell* 127, 125–37.
(7) Cardone, M.H. et al. (1998) *Science* 282, 1318–21.
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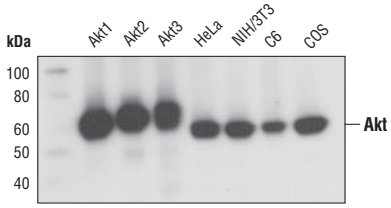
(10) Cantley, L.C. and Neel, B.G. (1999) *Proc Natl Acad Sci USA* 96, 4240–5.
(11) Vlahos, C.J. et al. (1994) *J Biol Chem* 269, 5241–8.
(12) Hajduch, E. et al. (2001) *FEBS Lett* 492, 199–203.
(13) Cross, D.A. et al. (1995) *Nature* 378, 785–9.
(14) Diehl, J.A. et al. (1998) *Genes Dev* 12, 3499–511.
(15) Gesbert, F. et al. (2000) *J Biol Chem* 275, 39223–30.
(16) Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245–52.
(17) Navé, B.T. et al. (1999) *Biochem J* 344 Pt 2, 427–31.
(18) Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648–57.
(19) Manning, B.D. et al. (2002) *Mol Cell* 10, 151–62.



Western blot analysis of recombinant Akt1, Akt2 and Akt3 proteins, and extracts from various cell lines, using ***Akt (pan) (C67E7) Rabbit mAb #4691****.*

#9270

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween®20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween®20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween®20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween®20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

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 per plate of 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 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.

MSDS

Material Safety Data Sheet (MSDS) for Antibodies

Cell Signaling TECHNOLOGY®

rev. 08/09/07

I. Identification:

Product name: Antibodies
Product Catalog Number: Includes antibodies within the following range of catalog numbers: 2000-5999, 7000-7999 and 9000-9999.
CAS number: None

Manufacturer Supplier: Cell Signaling Technology
3 Trask Lane
Danvers, MA 01923 USA
1-978-867-2300 TEL
1-978-867-2400 FAX
1-978-578-6737 Emergency Phone

II. Composition/Information on Ingredients:

This product is composed of antibodies in aqueous buffer solution. According to 29 CFR 1910.1200(d), hazardous ingredients at less than <1% and carcinogens at less than < 0.1% are considered non-hazardous. Any hazardous or carcinogenic ingredients exceeding these criteria are listed below.

This product may contain the following hazardous ingredients.

Ingredient	CAS#	Percent
Glycerol	56-81-5	50%

III. Hazard Identification:

Emergency Overview of Hazardous ingredient: Glycerol (CAS# 56-81-5)
Caution: Avoid contact and inhalation.
Target Organ: Kidneys.

NFPA Rating:

Health Rating:	1
Flammability Rating:	0
Reactivity Rating:	0

IV. First Aid Measures:

Inhalation: If inhaled, remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If swallowed and person is conscious, rinse out mouth with water. Get medical attention.
Skin Exposure: In case of contact, wash skin with soap and water.
Eye Exposure: In case of contact with eyes, immediately flush eyes water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Data not available.
Autoignition Temperature: Data not available.
Fire Extinguishing Media: Water spray, dry chemical, foam, or carbon dioxide.
Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.

VI. Accidental Release Measures:

Absorb liquid with an absorbent material. Transfer contaminated absorbent to a chemical waste container for disposal.

VII. Handling And Storage:

Avoid inhalation and contact with eyes and skin. Avoid prolonged or repeated exposure. Store at –20°C in tightly closed container.

VIII. Exposure Controls/Personal

Engineering Controls: Maintain adequate ventilation, eye wash and quick-drench facilities in work area.
Personal Protective Equipment: Lab coat, chemical resistant gloves and chemical safety glasses.
Occupational Exposure Limits: Data not available.

IX. Exposure Controls/Personal Protection:

Physical State:	Colorless liquid.
Odor:	Odorless.
Boiling Point:	Data not available.
Melting Point:	Data not available.
Volatile Organic Compound:	Data not available.
Solubility in water:	Readily miscible in water.

X. Stability and Reactivity:

Stability: Stable.
Hazardous Decomposition: May form carbon dioxide and carbon monoxide.
Conditions to avoid: Strong oxidizing agents

XI. Toxicological Information:

May cause skin irritation.
May be toxic if absorbed through skin or ingested.
May cause eye irritation.

Target Organs: Kidneys
Prolonged exposure may cause nausea, headache, and vomiting.

XII. Ecological Information:

Data not available.

XIII. Disposal Considerations:

Dispose of in accordance with federal, state and local environmental regulations.

XIV. Transport Information:

D.O.T.: This substance is considered non-hazardous for transport.
IATA: This substance is considered non-hazardous for air transport.

XV. Regulatory Information:

EU Regulation/Classification/Labeling Information: Not available for this product.

Chemical Inventory Status:
SARA Listed Component: None.
TSCA Listed Component: None.
Canada (WHMIS): DSL No, NDSL No.

XVI. Other Information:

This compound is sold only for research use by personnel familiar with chemicals and who are well trained in good laboratory habits, such as avoiding spills, keeping hands clean at all times and not rubbing eyes with hands while working in the laboratory.

This solution is sold only in microliter quantities for use in life sciences research. No other use is intended, and any other use may involve substantive hazards.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide for experienced personnel. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product. The burden of safe use of this material rests entirely with the user.