#7502 Store at –80°C

Protein Kinase

Akt1 Protein Kinase

☑ 100 assays

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ **www.cellsignal.com**

rev. 08/27/04

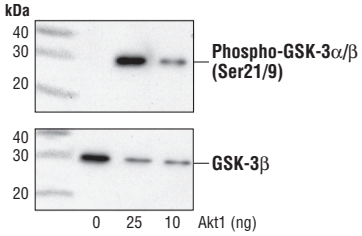
Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling the balance between survival and apoptosis (1–3). This protein kinase is activated by insulin and various growth and survival factors, and functions in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4), and by phosphorylation within the carboxy-terminus at Ser473. Akt promotes cell survival by inhibiting apoptosis through its ability to phosphorylate and inactivate several targets, including Bad (5), Forkhead transcription factors (6) and caspase-9. PTEN phosphatase is a major negative regulator of the PI3 kinase/Akt signaling pathway (7). LY294002 is a specific PI3 kinase inhibitor (8).

One of the essential functions of Akt is the regulation of glycogen synthesis through phosphorylation and inactivation of glycogen synthase kinase-3α and β (9,10). Akt may also play a role in insulin stimulation of glucose transport (9).

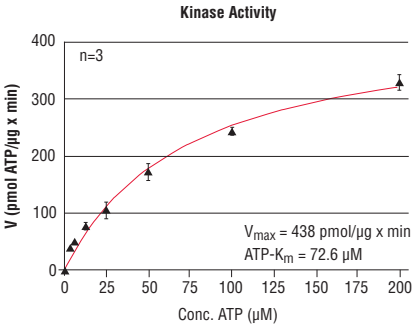
In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK3β-mediated phosphorylation and degradation of cyclin D1 (11), and by negatively regulating the cyclin-dependent kinase inhibitors p27 KIP (12) and p21 WAF1 (13). Akt also plays a critical role in cell growth by directly phosphorylating the mammalian target of rapamycin, mTOR (14), but more importantly through phosphorylation and inactivation of tuberin (TSC2), an mTOR inhibitor (15). 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 (16,17).

Description: ProQinase GmbH enzyme re-formulated by CST scientists for use in *in vitro* kinase activity assays. Active Akt1/PKB Protein Kinase is activated by PDK1 and contains human Akt1 residues 107-480. 10X Kinase Buffer #9802 is provided with the enzyme, and should be diluted 1:10 in deionized water.

Source/Purification: Human Akt1/PKB Protein Kinase fragment containing residues 107–480, activated by PDK1, was expressed by ProQinase GmbH using a baculovirus expression system. The fragment was amino-terminally fused to a GST-HIS_6-Thrombin cleavage site. The protein was then purified by one-step affinity purification using glutathione-agarose.



Kinase activity assay using Akt Kinase Assay Kit #9840: Varying amounts of Akt1 Protein Kinase were used to phosphorylate GSK-3 fusion protein #9278. Activated GSK-3 was detected by Western blot using Phospho-GSK-3α/β (Ser21/9) Antibody #9331 (upper), and total GSK-3β was detected using GSK-3β Antibody #9332.



Akt1 kinase activity was measured by ProQinase GmbH in a radioisotopic filtration assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg / 50 µl PEG20.000, Substrate: GSK3 (14-27), 5 µg / 50 µl, Recombinant Akt1: 100 ng / 50 µl.

Suggested Reaction Buffer: 1X Kinase Buffer: 25 mM Tris (pH 7.5), 5 mM β-Glycerolphosphate, 2 mM DTT, 0.1 mM Na_3VO_4, 10 mM $MgCl_2$. Supplement with 200 µM ATP.

Suggested Kinase Assay Condition: 1X Kinase Buffer, 200 µM ATP, 0.1 µM substrate, 25 ng Akt in a 100 µl reaction, incubate at 30°C for 30 minutes.

Note: Optimal incubation times and enzyme concentrations must be determined empirically for each particular substrate.

Storage: Supplied in 50 mM Tris-HCl, pH=8.0, 100 mM NaCL, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at -80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Phospho-Akt (Ser473) Antibody #9271

Phospho-Akt (Ser473) (193H12) Rabbit mAb #4058

Akt Antibody #9272

GSK-3 Fusion Protein #9278

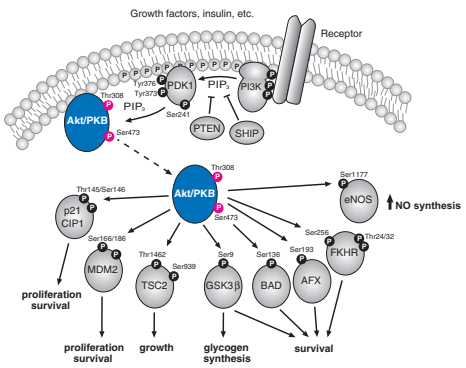
Phospho-GSK-3α/β (Ser21/9) Antibody #9331

Phospho-(Ser/Thr) Akt Substrate Antibody #9611

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Akt Kinase Assay Kit (Nonradioactive) #9840



ProQinase TOOLS & TESTS

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Protein Kinases distributed by Cell Signaling Technology are produced by ProQinase GmbH. Please direct all inquiries to: drugdiscovery@cellsignal.com

References:

(1) Franke, T.F. (1997) *Cell* 88, 435–437.
(2) Burgering, B.T. and Coffer, P.J. (1995) *Nature* 376, 599–602.
(3) Franke, T. F. et al. (1995) *Cell* 81, 727–736.
(4) Alessi, D. R. et al. (1996) *EMBO J.* 15, 6541–6551.
(5) Cardone, M. H. et al. (1998) *Science* 282, 1318–1321.
(6) Brunet, A. et al. (1999) *Cell* 96, 857–868.
(7) Cantley, L. C. et al. (1999) *Proc. Natl. Acad. Sci. USA* 96, 4240–4245.
(8) Vlahos, C. et al. (1994) *J. Biol. Chem.* 269, 5241–5248.
(9) Hajduch, E. et al. (2000) *FEBS Lett.* 492, 199–203.
(10) Cross, D. A. et al. (1995) *Nature* 373, 785–789.
(11) Diehl, et al. (1998) *Genes Dev.* 12, 3499–3511.
(12) Gesbert, F. et al. (2000) *J. Biol. Chem.* 273, 39223–39230.
(13) Zhou, B. P. et al. (2001) *Nat. Cell Biol.* 3, 245–252.
(14) Nave, B. T. et al. (1999) *Biochem. J.* 344, 427–431.
(15) Manning, B. D. et al. (2000) *Mol. Cell* 4, 648–657.
(16) Manning, B. D. et al. (2002) *Mol. Cell* 10, 151–162.
(17) Inoki, K. et al. (2002) *Nat. Cell Biol.* 4, 648–657.

Akt Reactivity ELISA/Heterogeneous (Solid Phase) Assay Protocol

Solutions and Reagents

- GSK-3 Fusion Protein #9278
- Carbonate-Bicarbonate Buffer Pack (Pierce #28382)
  0.2 M sodium carbonate-bicarbonate buffer, pH 9.4 when pouch contents dissolved in a final volume of 500 ml degassed, deinozed water.
- Wash Buffer:
  1X TBS, 0.1% Tween 20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Blocking Buffer:
  3% BSA in TBS/T
- Kinase Buffer (10X) #9802
- ATP (10 mM) #9804
- Akt1/PKB Protein Kinase #9274
- Phospho-(Ser/Thr) Akt Substrate Antibody #9611
- DELFIA Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #1244-100)
- DELFIA Enhancement Solution (PerkinElmer Life Sciences #1244-105)

(DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol

Note: Akt reactivity can also be demonstrated by homogeneous (solution) assay using Alphascreen.

1. Coat a 96-well plate with GSK-3 Fusion Protein, 100 µl per well, 0.1 uM in carbonate buffer. Incubate at room temperature for 3 hours.
2. Wash plate three times 200 µl/well with TBS/T.
3. Block plate with 200 µl/well 3% BSA in TBS/T for 1 hour at room temperature. Wash plate three times with TBS/T. (May leave dry plate at 4°C for 1–2 months if desired.)
4. Set incubator to 30°C.
5. Set up kinase reaction in tubes on ice, starting with the water, as follows:
   a. 10 µl Kinase Buffer (10X)
   b. 2 µl ATP (200 µM)
   c. 1 µl of Akt1/PKB Protein Kinase [10 units/µl]
   d. Remaining microliters of sterile water to equal final volume of 100 µl/rxn.
6. Transfer reactions to a 96-well plate and incubate at 30°C for 30 minutes.
7. Wash three times with TBS/T.
8. Add 8 ng/well of Phospho-(Ser/Thr) Akt Substrate Antibody, diluted in 100 µl/well blocking buffer. Incubate at 37°C for 30 minutes.
9. Wash three times with TBS/T.
10. Add 67 ng/well DELFIA Europium-labeled Anti-rabbit IgG, diluted in 100 ul/well blocking buffer. Incubate at 37°C for 30 minutes.
11. Wash six times with TBS/T.
12. Add 200 µl enhancement solution and incubate at 37°C for 15 minutes. Read plate with a multilabel reader.