

# Akt2 Kinase

✓ 5 µg

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant human Akt2 kinase (Ala107-Glu481), supplied as a GST fusion protein.

**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), c-Raf (7) and caspase-9. PTEN phosphatase is a major negative regulator of the PI3 kinase/Akt signaling pathway (8). LY294002 is a specific PI3 kinase inhibitor (9).

One of the essential functions of Akt is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3 $\alpha$  and  $\beta$  (10,11). Akt may also play a role in insulin stimulation of glucose transport (10).

In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3 mediated phosphorylation and degradation of cyclin D1 (12) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (13) and p21 Waf1 (14). Akt also plays a critical role in cell growth by directly phosphorylating the mammalian target of rapamycin, mTOR (15), but more importantly through phosphorylation and inactivation of tuberlin (TSC2), an mTOR inhibitor (16). 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 (17,18).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human Akt2 (Ala107-Glu481) (GenBank Accession No. NM\_001626.2) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

**Quality Control:** The theoretical molecular weight of the GST-Akt2 fusion protein is 73 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Silver stain and Western blot [Fig.1]. Akt2 kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure Akt2 activity using HTScan™ Akt2 Kinase Assay Kit #7504 [Fig.3].

#### Background References:

- (1) Franke, T.F. (1997) *Cell* 88, 435–437.
- (2) Burgering, B.T. and Coffey, 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) Zimmerman, S. et al. (1999) *Science* 286, 1741–1744.
- (8) Cantley, L.C. et al. (1999) *Proc. Natl. Acad. Sci. USA* 96, 4240–4245.
- (9) Vlahos, C. et al. (1994) *J. Biol. Chem.* 269, 5241–5248.
- (10) Hajduch, E. et al. (2000) *FEBS Lett.* 492, 199–203.
- (11) Cross, D.A. et al. (1995) *Nature* 373, 785–789.
- (12) Diehl, J.A. et al. (1998) *Genes Dev.* 12, 3499–3511.
- (13) Gesbert, F. et al. (2000) *J. Biol. Chem.* 275, 39223–39230.
- (14) Zhou, B.P. et al. (2001) *Nat. Cell Biol.* 3, 245–252.
- (15) Nave, B.T. et al. (1999) *Biochem. J.* 344, 427–431.
- (16) Manning, B.D. et al. (2000) *Mol. Cell* 4, 648–657.
- (17) Manning, B.D. et al. (2002) *Mol. Cell* 10, 151–162.
- (18) Inoki, K. et al. (2002) *Nat. Cell Biol.* 4, 648–657.

**Storage:** Enzyme is 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 on ice during use.

Avoid repeated freeze-thaw cycles.

#### Companion Products:

HTScan™ Akt2 Kinase Assay Kit #7504

eNOS (Ser1177) Biotinylated Peptide #1133

Phospho-eNOS (Ser1177) (C9C3) Rabbit mAb #9570

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

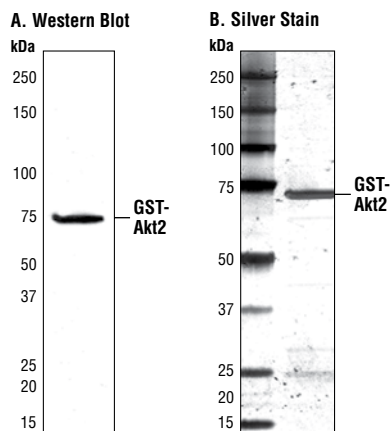


Figure 1. The purity of the GST-Akt2 fusion protein was analyzed using SDS/PAGE followed by anti-Akt2 Western blot (A) or Silver stain (B).

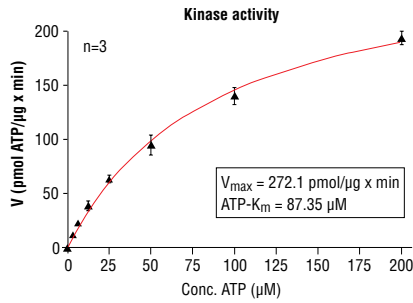


Figure 2. Akt2 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl<sub>2</sub>, 3 mM MnCl<sub>2</sub>, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: R11-GSK3 (14-27), 5 µg/50 µl and 100 ng/50 µl Recombinant Akt2.

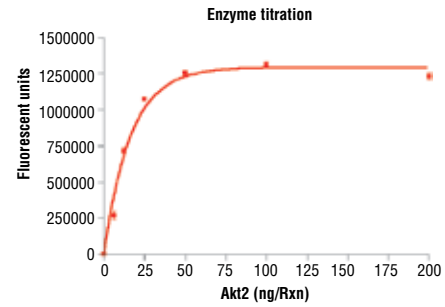


Figure 3. Dose dependence curve of Akt2 kinase activity: DELFIA® data generated using Phospho-eNOS (Ser1177) (C9C3) Rabbit mAb #9570 to detect phosphorylation of substrate peptide (#1133) by Akt2 kinase. In a 50 µl reaction, increasing amounts of Akt2 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIATM is a registered trademark of PerkinElmer, Inc.)

## Protocol for Akt2 Kinase Assay

**\*IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

### Kinase

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

### A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-eNOS (Ser1177) (C9C3) Rabbit mAb #9570
5. Kinase Buffer (10X) #9802
6. ATP (10 mM) #9804
7. eNOS (Ser1177) Biotinylated Peptide #1133
8. DELFIA® Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
9. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
10. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

*DELFIA® is a registered trademark of PerkinElmer Life Sciences*

### B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH<sub>2</sub>O to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µM).
2. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM MgCl<sub>2</sub>, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH<sub>2</sub>O to make 2.5 ml 4X reaction buffer.
5. Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail ([enzyme]=4.0 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

### Final Assay Conditions for a 50 µl Reaction

- 25 mM Tris-HCl (pH 7.5)
  - 10 mM MgCl<sub>2</sub>
  - 5 mM β-glycerophosphate
  - 0.1 mM Na<sub>3</sub>VO<sub>4</sub>
  - 2 mM DTT
  - 200 µM ATP
  - 1.5 µM peptide
  - 50 ng Akt2 Kinase
8. Incubate reaction plate at room temperature for 30 minutes.
  9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
  10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH<sub>2</sub>O/well and incubate at room temperature for 60 minutes.
  11. \*Wash three times with 200 µl/well PBS/T.
  12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.  
**Please note:** This protocol was validated using a eNOS (Ser1177) Biotinylated Peptide and Phospho-eNOS (Ser1177) Antibody diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
  13. Incubate at 37°C for 120 minutes.
  14. \*Wash three times with 200 µl/well PBS/T.
  15. Dilute Europium labeled secondary antibody 1:1000 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
  16. Incubate at room temperature for 30 minutes.
  17. \*Wash five times with 200 µl/well PBS/T.
  18. Add 100 µl/well DELFIA® Enhancement Solution.
  19. Incubate at room temperature for 5 minutes.
  20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.  
Email: [drugdiscovery@cellsignal.com](mailto:drugdiscovery@cellsignal.com)