

#7535 Store at -80°C

Akt1 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 full-length human Akt1 kinase, supplied as a GST fusion protein.

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 tuberlin (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).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human Akt1 (Met1-Ala480) (GenBank Accession No. NM_005163) 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 Akt1 fusion protein is 85 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Akt1 kinase activity was determined using a radiometric assay [Fig.2].

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.
Avoid repeated freeze-thaw cycles.

Companion Products:
HTScan® Akt1 Kinase Assay Kit #7501
Phospho-eNOS (Ser1177) (C9C3) Rabbit mAb #9570
eNOS (Ser1177) Biotinylated Peptide #1133
ATP (10 mM) #9804
Kinase Buffer (10X) #9802
Staurosporine #9953

Background References:

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- (12) Hajdich, E. et al. (2001) *FEBS Lett* 492, 199-203.
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- (16) Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245-52.
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- (18) Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648-57.
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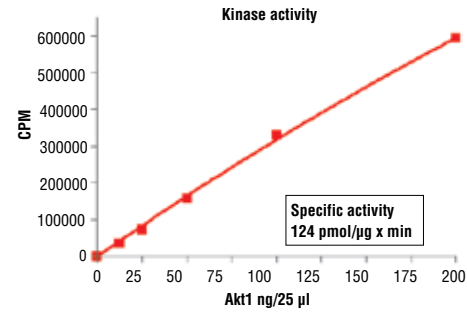


Figure 2. Akt1 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl₂, 0.05 mM DTT, 50 µM ATP. Substrate: Akt-substrate peptide 200 ng/µL, and variable amount of recombinant Akt1.

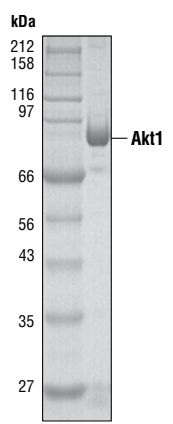


Figure 1. The purity of the Akt1 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Protocol for Akt1 Kinase Assay

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. Kinase Buffer (10X)**
50 mM MOPS, pH 7.2
25 mM β -glycerophosphate
10 mM EGTA
4 mM EDTA
50 mM $MgCl_2$
0.5 mM DTT
- 2.** ATP (10 mM) #9804
- 3.** ^{32}P - γ ATP
- 4.** Akt-substrate peptide (0.5 μ g/ μ l)

B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
- 2.** Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
- 3.** Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- 4.** Dilute Akt1 protein to 20 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10 μ l diluted Akt1 kinase solution, 10 μ l Akt-substrate peptide (0.5 μ g/ μ l), and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

- 5 mM MOPS, pH 7.2
 - 2.5 mM β -glycerophosphate
 - 1 mM EGTA
 - 0.4 mM EDTA
 - 5 mM $MgCl_2$
 - 0.05 mM DTT
 - 200 ng/ μ l Akt-substrate peptide
- 6.** After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
 - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
 - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
 - 9.** Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.