

## AKT3 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPA18

Lot No. A269-1  
5 µg 0.1 µg/µl

### Background:

AKT3 or Protein Kinase B  $\gamma$  (PKB  $\gamma$ ) is a serine/threonine kinase that is a member of the AKT family. AKT3 is activated in cells exposed to diverse stimuli such as hormones, growth factors, and extracellular matrix components (1). AKT3 phosphorylates and regulates the function of many cellular proteins involved in processes that include cellular metabolism, survival/apoptosis, and proliferation. Recent evidence indicates that AKT3 is frequently overexpressed in many types of human cancers including breast and prostate (2).

### Product Description:

Recombinant full-length human AKT3 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM\_005465.

### Gene Aliases:

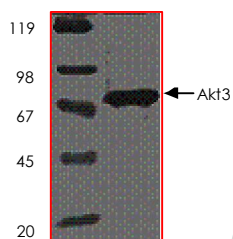
PKBG; PRKBG; STK-2; RAC-gamma; RAC-PK-gamma

### Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

### Purity & Molecular Weight:

The purity was determined to be >90% by densitometry. Approx. MW 85kDa.



### Storage:

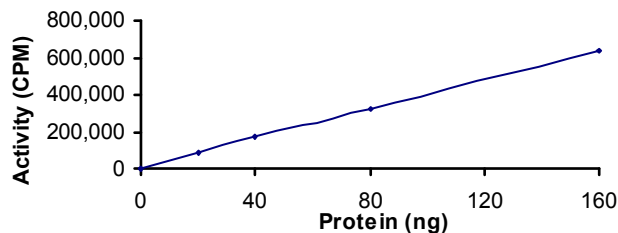
Store product at  $-70^{\circ}\text{C}$ . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

### Stability:

Unopened vial at  $-70^{\circ}\text{C}$ , 1 year from date of shipment.

**Specific Activity:**

The specific activity was determined to be 206 nmol/min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25  $\mu$ L reaction consisting of 5  $\mu$ L of 5 X Kinase Assay Buffer, 5  $\mu$ L of 1 mg/ml the Substrate Solution, 10  $\mu$ L of diluted kinase and 5  $\mu$ L of 250  $\mu$ M ATP solution containing [ $\gamma$ - $^{32}$ P] ATP (0.167  $\mu$ Ci/ $\mu$ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20  $\mu$ L of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

**Substrate Solution:**

AKT-sub synthetic peptide substrate (RPRAATF) diluted in distilled H<sub>2</sub>O to a final concentration of 1 mg/ml.

**5 X Kinase Assay Buffer:**

25mM MOPS, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**References:**

1. Coffey, P.J. et al: Protein kinase B ( $\epsilon$ -Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. *Biochem J.* 1998 Oct 1; 335 (Pt 1): 1-13.
2. Anderson, K.E. et al: Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B. *Curr Biol.* 1998 Jun 4;8(12): 684-91.

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