

Product: AKT3, Active

Catalog #: 02-2003

Amount: 5µg

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.

Storage and Stability

Store product at -70°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.

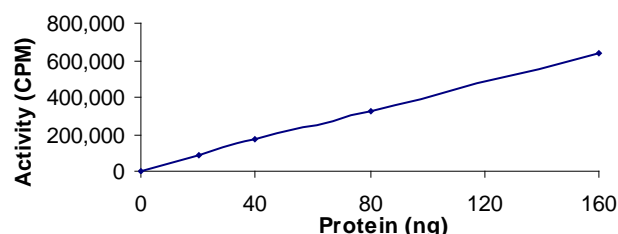
Scientific Background

AKT 3 or Protein Kinase B γ (PKB γ) is a serine/threonine kinase that is a member of the AKT family. AKT 3 is activated in cells exposed to diverse stimuli such as hormones, growth factors, and extracellular matrix components (1). AKT 3 phosphorylates and regulates the function of many cellular proteins involved in processes that include cellular metabolism, survival/apoptosis, and proliferation. Recent evidence indicates that AKT 3 is frequently overexpressed in many types of human cancers including breast and prostate (2).

References

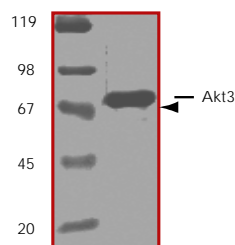
1. Coffey, P.J. et al: Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. *Biochem J.* 1998 Oct 1; 335 (Pt 1): 1-13.
2. Anderson, K.E. et al: Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B. *Curr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

Specific Activity



The specific activity of AKT3 was determined to be **206 nmol/min/mg** as per activity assay protocol.

Purity



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

AKT3, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	02-2003
Quantity	5µg
Specific Activity	206 nmol/min/mg
Specific Lot Number	A269-1
Purity	>90%
Format	5µg in 50µl
Concentration	0.1µg/µl
Stability	1yr At -70°C from date of shipment
Storage & Shipping	Store product at -70°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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: 02-2003)

Active AKT3 (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K25-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active AKT3 for optimal results).

Kinase Dilution Buffer, pH 7.2 (Catalog #: K25-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 5% glycerol solution.

Kinase Assay Buffer I, pH 7.2 (Catalog #: K01-09)

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM , 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³²P]-ATP Assay Cocktail

Prepare 250µM [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³²P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at –.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200µl aliquots at –.

Substrate

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

Assay Protocol

- Step 1. Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active AKT3, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1. 10µl of diluted Active AKT3 (Catalog #02-2003).
 - Component 2. 5µl of 1mg/ml stock solution of substrate
 - Component 3. 5µl distilled H₂O (4°C)
- Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5. Initiate the reaction by the addition of 5µl [³²P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³²P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [³²P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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