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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
A17-10G -05	5 µg
A17-10G -10	10 µg

AKT2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # A17-10G

Lot # M2946-7

Product Description

Recombinant full-length human AKT2 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_001626.

Gene Aliases

PRKBB; PKBBETA; RAC-BETA

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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.

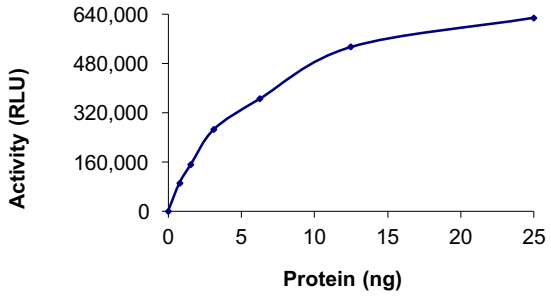
Scientific Background

AKT2 or Protein Kinase B β (PKBβ) is a serine/threonine kinase that is a member of the AKT family. AKT2 like the other AKT members is activated in cells in response to diverse stimuli such as hormones, growth factors and extracellular matrix components and is involved in glucose metabolism, transcription, survival, cell proliferation, angiogenesis, and cell motility (1). The PI3K generates phosphatidylinositol-3, 4, 5-trisphosphate (PIP_3), a lipid second messenger essential for the translocation of AKT2 to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase-1 (PDK-1) (2).

References

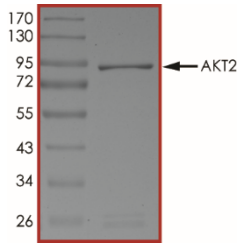
1. Coffer, PJ. et al: Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. Biochem J. 1998 Oct 1; 335 (Pt 1):1-13.
2. Anderson, KE. et al: Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B. Curr Biol.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

Specific Activity



The specific activity of AKT2 was determined to be **80 nmol /min/mg** as per activity assay protocol, and was equivalent to **52 nmol/min/mg** as per radiometric assay.

Purity



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

AKT2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	A17-10G
Specific Activity	80 nmol/min/mg
Lot #	M2946-7
Purity	>90%
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 #: A17-10G)

Active AKT2 (0.1μg/μl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) 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 AKT2 for optimal results).

ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM $MgCl_2$ and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250μM.

Kinase Dilution Buffer IX (1x) (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50μM.

Substrate (Catalog #: A05-58B)

Modified AKT Substrate peptide (modified-CKRPRAASFAE) was diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

The AKT2 assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the AKT2 reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the Active AKT2, Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 μL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1x), in a pre-chilled 96-well plate.

Step 2. Prepare a substrate/ATP mixture as follows (25 μM example):

Component	Amount (μL)	Component	Amount (μL)
10μM ATP Solution	1	Substrate at 1mg/mL	80
Kinase Assay Buffer III (5x)	79		

Step 3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5μL:

Component 1. 3μl of diluted the Active AKT2 (Catalog # A17-10G).
Component 2. 2μl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

Step 4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1x).

Step 5. Incubate at ambient temperature for 40 minutes.

Step 6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5μl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.

Step 7. Then add 10μl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

Step 8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).

Step 9. Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

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

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)

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