

AKT1 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPA16

Lot No. A320-1
5 µg 0.1 µg/µl

Background:

AKT1/PKB α is a serine/threonine kinase that belongs to the AKT family. AKT1 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). AKT1 is frequently overexpressed and active in many types of human cancers including cancers of colon, breast, brain, pancreas and prostate as well as lymphomas and leukemias (2).

Product Description:

Recombinant full-length human AKT1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_005163.

Gene Aliases:

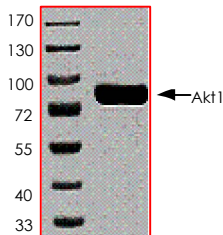
PKB; RAC; PRKBA; MGC99656; RAC-ALPHA

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:

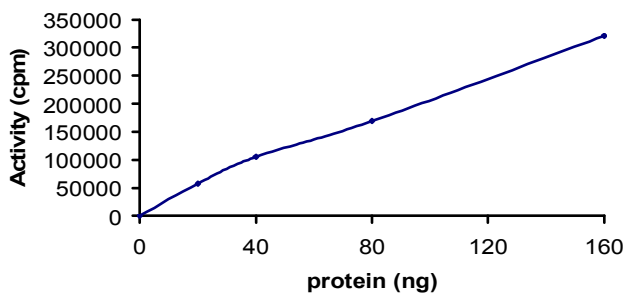
Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

Unopened vial at -70°C , 1 year from date of shipment.

**Specific Activity:**

The specific activity was determined to be 124 nmol/min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of AKT1 in a 25 μ L reaction consisting of 5 μ L of 5 X Kinase Assay Buffer, 5 μ L of 1 mg/ml the Substrate Solution, 10 μ L of diluted AKT1 and 5 μ L of 250 μ M ATP solution containing [γ - 32 P] ATP (0.167 μ Ci/ μ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 μ 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 H₂O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

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.

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