

Product Specification

Akt2, active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7702-5
 Lot #: _____
 Aliquot size: 5- μ g protein in 50 μ l
 Specific activity: 46 nmol/min/mg

Quality Control Analysis

Activity assessment

Akt2 protein (100 ng/ μ l concentration) was diluted to 50ng/ μ l with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT and 50ng/ μ l BSA), followed by 2-fold serial dilutions, and then the 10 μ l diluted proteins were used to phosphorylate the Akt/SGK substrate peptide (RPRAATF) using the following assay condition:

- 10 μ l diluted Akt2 protein
- 10 μ l Akt/SGK substrate peptide (1 mg/ml stock)
- 5 μ l [³²P] ATP mixture (250 \square M ATP, 0.16 μ Ci/ μ l in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30⁰C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 μ l of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

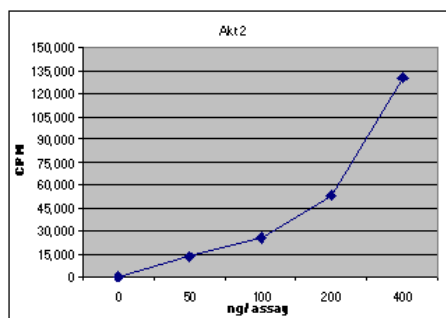


Fig. 1 Akt2 activity assay

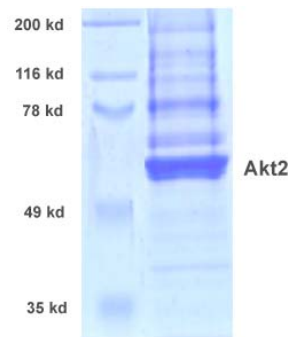


Fig. 2 Akt2 protein gel

Purity assessment

3 μ g of Akt2 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the Akt2 product, and the major band was at ~58 kDa (Fig. 2).

Product Description

Recombinant full-length human Akt2 containing N-terminal His tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_001626.

This material is sold for research purposes only.

Specific Activity

46 nmol phosphate incorporated into Akt/SGK substrate (RPRAATF) per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μM ATP and total of 0.83 μCi/μl P-32.

Formulation

Recombinant proteins in storage buffer (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).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

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 (PIP₃), 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) and phosphoinositide-dependent kinase-2. Akt1 has numerous cellular substrates including proteins, which promote the inhibition of apoptosis such as the Forkhead transcription factors and the Bcl-2 family member Bad (3).

References

1. Coffey PJ, Jin J, Woodgett JR. *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, Coadwell J, Stephens LR, Hawkins PT. *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.
3. Sen P, Mukherjee S, Ray D, Raha S. *Involvement of the Akt/PKB signaling pathway with disease processes*. *Mol Cell Biochem*. 2003 Nov; 253(1-2): 241-6.