

Product Specification

Akt3, active

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

Catalog #: 7703-5
 Lot #: _____
 Aliquot size: 5 µg protein in 50 µl
 Specific activity: 206 nmol/min/mg

Quality Control Analysis

Activity assessment

Akt3 protein (0.1 µg/µl concentration) was diluted to 20ng/µl 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), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Akt substrate peptide (RPRAATF) using the following assay condition:

- 10 µl diluted Akt3 protein
- 10 µl Akt substrate peptide (1 mg/ml stock)
- 5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30^oC and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl 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.

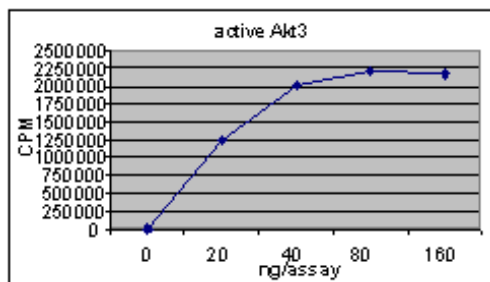


Fig. 1 Akt3 activity assay

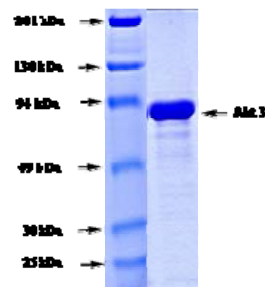


Fig. 2 Akt3 protein gel

Purity assessment

3 µg of Akt 3 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >92% purity of the Akt3 product, and the band was at ~84 kDa (Fig. 2).

Product Description

Recombinant full-length human Akt 3 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_005465.

This material is sold for research purposes only.

Specific Activity

206 nmol phosphate incorporated into Akt substrate peptide per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μ M ATP and total of 0.83 μ Ci/ μ l 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

Akt 3 or Protein Kinase B γ (PKB γ) is a serine/threonine kinase that is a member of the Akt family. In mammals, the Akt family comprises of three highly homologous members known as Akt1 (PKB), Akt2 (PKB), and Akt 3 (PKB γ). Akt 3 like the other family members is activated in cells exposed to diverse stimuli such as hormones, growth factors, and extracellular matrix components. The activation mechanism remains to be fully characterized but occurs downstream of phosphoinositide 3-kinase (PI3K). PI3K generates phosphatidylinositol-3,4,5-trisphosphate (PIP₃), a lipid second messenger essential for the translocation of Akt family members to the plasma membrane where they are phosphorylated and activated by phosphoinositide-dependent kinase-1 (PDK-1) and phosphoinositide-dependent kinase-2 (PDK-2 possibly ILK). Akt 3 like the other family members 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. Although the mechanisms have not yet been fully characterized, increased expression and activation of Akt is believed to promote cell proliferation and survival thereby contributing to cancer progression.