

## Product Specification

### **Akt1, active**

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

Catalog #: 7701-5  
 Lot #: \_\_\_\_\_  
 Aliquot size: 5- $\mu$ g protein in 50  $\mu$ l  
 Specific activity: 124 nmol/min/mg

### **Quality Control Analysis**

#### Activity assessment

Akt1 protein (100 ng/ $\mu$ l concentration) was diluted to 20ng/ $\mu$ l with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM  $\beta$ -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 30 mM MgCl<sub>2</sub>, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10 $\mu$ l diluted proteins were used to phosphorylate the Akt substrate peptide (RPRAATF) using the following assay condition:

- 10  $\mu$ l diluted Akt1 protein
- 10  $\mu$ l Akt substrate peptide (1 mg/ml stock)
- 5  $\mu$ l [<sup>32</sup>P] ATP mixture (250  $\mu$ M ATP, 0.16  $\mu$ Ci/ $\mu$ l in 4x assay dilution buffer)

The various reaction components, except [<sup>32</sup>P] ATP, were incubated at 30°C and the reaction started by the addition of [<sup>32</sup>P] ATP. After 15 minutes, the reaction was terminated by spotting 20  $\mu$ 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.

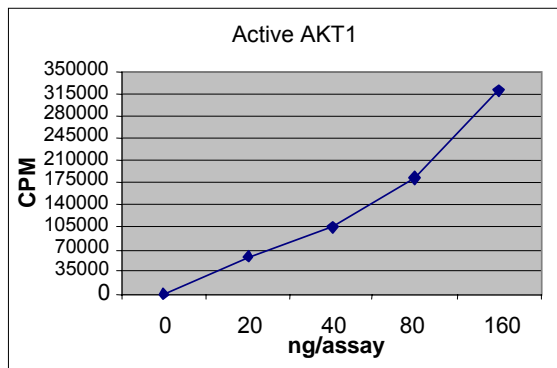


Fig. 1 Akt1 activity assay

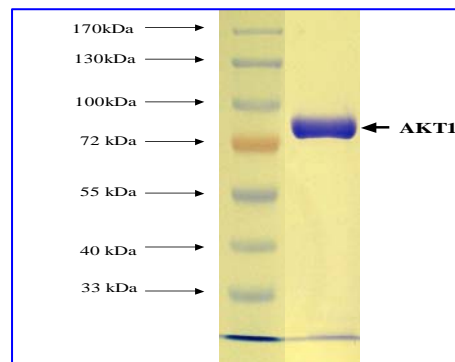


Fig. 2 Akt1 protein gel

#### Purity assessment

4  $\mu$ g of Akt1 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the Akt1 product, and the band was at ~85 kDa (Fig. 2).

## Product Description

Recombinant full-length human Akt1 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM\_005163.

This material is sold for research purposes only.

## Specific Activity

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

Akt1 or Protein Kinase B  $\alpha$  (PKB $\alpha$ ) 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). The PI3K generates phosphatidylinositol-3,4,5-trisphosphate (PIP<sub>3</sub>), a lipid second messenger essential for the translocation of Akt1 to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase-1 (PDK-1) (2) and phosphoinositide-dependent kinase-2 (possibly ILK) (3). 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 (4). In addition, the cyclin dependent kinase inhibitors are substrates of Akt which when phosphorylated relinquish their inhibitory influence on cell cycle progression. Akt also mediates many of the stimulatory effects of insulin on glucose metabolism through deactivation of glycogen synthase kinase, activation of phosphofructo-kinase, and modulation of glucose transporter activity. Akt1 has been shown to play a role in human cancers (4). It 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 (5). Akt1 plays an important role in cancer cell survival and proliferation thereby contributing to cancer progression.

## References

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3. Persad S, Attwell S, Gray V, Mawji N, Deng JT, Leung D, Yan J, Sanghera J, Dedhar S. *Regulation of protein kinase B/Akt-serine 473 phosphorylation by integrin-linked kinase: critical roles for kinase activity and amino acids arginine 211 and serine 343*. J Biol Chem. 2001 Jul 20; 276(29): 27462-9.
4. 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.
5. Nicholson KM, Anderson NG. *The protein kinase B/Akt signalling pathway in human malignancy*. Cell Signal. 2002 May; 14(5): 381-95.