Recombinant Human Active Akt1 Protein, CF

Catalog Number: 1775-KS-010

Lot Number: 1757167

SPECIFICATIONS AND USE

Source	Recombinant full-length human Akt1 was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_005163.
Molecular Mass	The approximate molecular weight is 85 kDa.
Purity	The purity was determined to be >90% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 10 mM Glutathione, 0.1 mM EDTA, 0.1 mM PMSF, 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of Akt1 was determined to be 75 nmol/min/mg as per Activity Assay Protocol and was equivalent to 127 nmol/min/mg as per radiometric assay.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

Akt1/PKBα 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 over-expressed and active in many types of human cancers including cancers of colon, breast, brain, pancreas and prostate as well as lymphomas and leukemias (2).

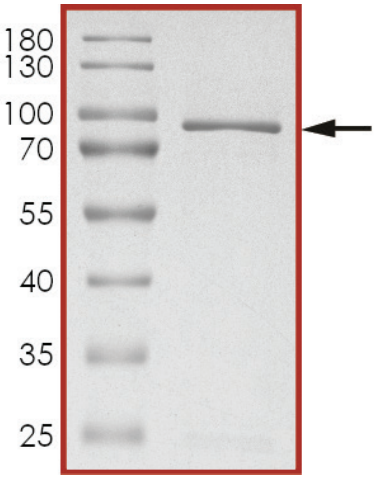

Figure 1: The purity of Akt1 was determined to be >90% by densitometry. Approximately MW 85kDa.

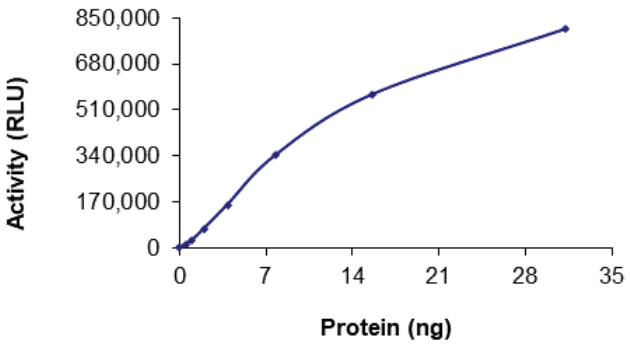

Figure 2: The specific activity of Akt1 was determined to be 75 nmol/min/mg as per Activity Assay Protocol and was equivalent to 127 nmol/min/mg as per radiometric assay.

REFERENCES

1. Coffer, P.J. *et al.* (1998) Biochem J. **335**:1.
2. Anderson, K.E. *et al.* (1998) Curr Biol. **8**:684.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active Akt1 (0.1 µg/µL) diluted with Kinase Dilution Buffer IX (1X) and assayed as outlined in Figure 2.
Note: *These are suggested working dilutions and it is recommended that the researcher perform a serial dilution for optimal results.*

Kinase Assay Buffer III (5X) - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$ and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer IX (1X) - Kinase Assay Buffer III 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 50 µM.

ADP-Glo™ Kinase Assay Kit- 10 mM of ATP Solution, 10 mM of ADP Solution, ADP-Glo Reagent, Kinase Detection Reagent.

Substrate - AKT (PKB) synthetic peptide substrate (CKRPRAASFAE) diluted in 25 mM Tris-HCl Buffer (pH 7.5) to a final concentration of 1 mg/mL.

ASSAY PROCEDURE

The Akt1 assay is performed using the ADP-Glo Kinase Assay Kit which quantifies the amount of ADP produced by the Akt1 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.

1. Thaw the Active Akt1, Kinase Assay Buffer lll (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.
2. Prepare a Substrate/ATP mixture as follows (25 µM example):

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

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

Component 1	3 µL of diluted Active Akt1.
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. *This initiates the reaction.*

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).
5. Incubate at ambient temperature for 40 minutes.
6. After the 40 minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
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)