

Certificate of Analysis

Akt1/PKB α , active

(Recombinant enzyme expressed in Sf21 cells)

Item # 14-276, 14-276-K, 14-276M

Parent Lot # D9BN042U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, full length, human Akt1, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Activated using PDK1 (cat# 14-452) and MAPKAPK2 (cat# 14-337). Purity 97.1% by SDS-PAGE and Coomassie blue staining. MW = 59.1kDa.

Formulation: 1.803mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 150mM imidazole, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.02% Brij-35, 270mM sucrose. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Specific Activity (Parent lot# D9BN042U): 674U/mg, where one unit of Akt1 activity is defined as 1nmol phosphate incorporated into 30 μ M modified Crosstide (GRPRTSSFAEGKK) per minute at 30°C with a final ATP concentration of 100 μ M.

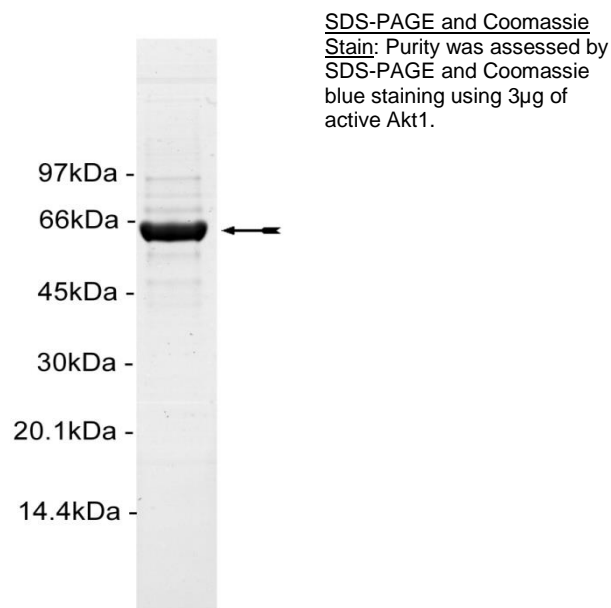
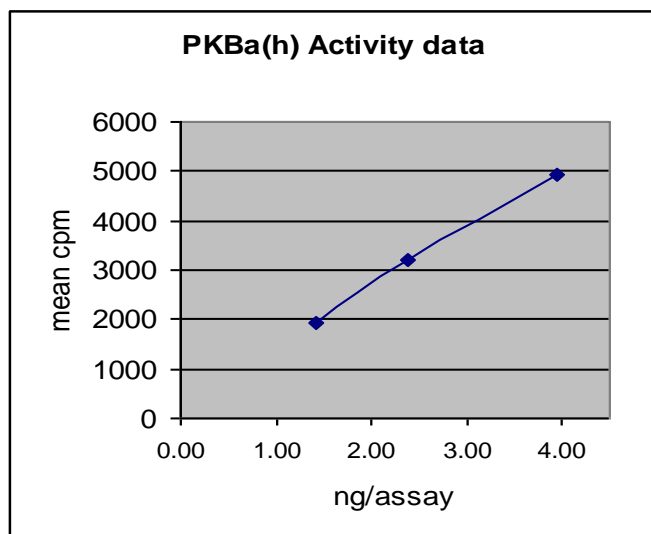
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.43–3.94ng of this lot of enzyme phosphorylated 30 μ M modified Crosstide (GRPRTSSFAEGKK) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.

MS Tryptic Fingerprint: Confirmed identity as PKB alpha with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH, pH7.0, 1mM EDTA.
- 2. Modified Crosstide (GRprtSSFAEGKK):** Use a final assay concentration of 30 μ M. Make a 300 μ M stock. Add 2.5 μ l of stock per assay point.
- 3. Akt1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.43–3.94ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **modified crosstide (GRprtSSFAEGKK)**.
3. Add **2.5 μ l (1.43–3.94ng) Akt1, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30 % phosphoric acid.

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Akt1 Sequence Information

<u>Protein</u>	Human Akt1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M29 of the recombinant protein is equivalent to M1 of human Akt1
<u>Accession number</u>	GenBank M63167. The recombinant protein contains the amino acid substitution S478G with respect to this accession number. This is reported in EMBL BE 206796.

Recombinant Akt1 amino acid sequence:

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1 MSFFHHHHHH DFDIPTTENL YFQGAMGSMS DVAIVKEGWL HKRGEYIKTW RPRYFLLKND
61 GTFIGYKERP QDVDQREAPL NNFSVAQCQL MKTERPRPNT FIIRCLQWTT VIERTFHVET
121 PEEREWTTA IQTVADGLKK QEEEEMDFRS GSPSDNSGAE EMEVSLAKPK HRVTMNEFEY
181 LKLLGKGTFG KVILVKEKAT GRYYAMKILK KEVIVAKDEV AHTLTENRVL QNSRHPFLTA
241 LKYSFQTHDR LCFVMEYANG GELFFHLSRE RVFSEDRARF YGAEIVSALD YLHSEKNVVY
301 RDLKLENLML DKDGHKIDT FGLCKEIKD GATMKTFCGT PEYLAPEVLE DNDYGRAVDW
361 WGLGVVYEM MCGRLPFYNQ DHEKLFELIL MEEIRFPRTL GPEAKSLLSG LLKKDPKQRL
421 GGGSEDAKEI MQHRFFAGIV WQHVYEKCLS PPFKQVTSE TDTRYFDEEF TAQMIIITPP
481 DQDDSMCEVD SERRPHFPQF SYSASGTA
  
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Recombinant Akt1 nucleotide sequence:

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1 atgtcgttct tccatcacca tcaccatcac gatttcgata tcccaacgac cgaaaacctg
61 tattttcagg ggcctatggg atccatgagc gacgtggcta ttgtgaagga gggttggctg
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Reviewed and approved by site quality representative.

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