

Certificate of Analysis

Akt1/PKB α , unactive

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-279, 14-279-K, 14-279M

Parent Lot # D8SN004U

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/PKB α , unactive, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 67.7% by SDS-PAGE and Coomassie blue staining. MW = 59kDa.

Formulation: 2mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol.

Storage and Stability: On receipt of material store at -20°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# D8SN004U): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with PDK1 and MAPKAP-K2.

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

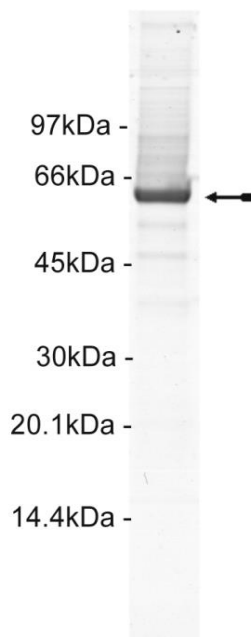
Quality Control Testing

Activation Assay: 0.2mg/ml unactive Akt1/PKB α was activated using 0.02mg/ml PDK1 (Catalogue# 14-452) and 25U/ml MAPKAP-K2 (Catalogue# 14-337) and the increased activity against modified crosstide determined. The activation and assay are described on page two. Results of this assay are shown below.

MS Tryptic Fingerprint: Confirmed identity as Akt1 with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of Akt1/PKB α , unactive.

Active PDK1	Active MAPKAP-K2	Unactive Akt1/PKB α	Mean cpm	Comments
None	None	5 μ g	5	Background
0.5 μ g	625mU	5 μ g	62529	Kinase activity



Certificate of Analysis

Kinase Cascade Assay Protocol

Stock Solutions:

- Akt1/PKB α Activation Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 2mM NaCl, 1% 2-mercaptoethanol.
- 5 x Assay Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- Akt1/PKB α , inactive:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA to 2mg/ml. Use 2.5 μ l per assay point.
- PDK1, active (Catalogue#14-452):** Use at a final assay concentration of 0.02mg/ml. Prepare a 0.2mg/ml stock and add 2.5 μ l of stock per assay point.
- MAPKAP-K2, active (Catalogue# 14-337):** Use at a final assay concentration of 25U/ml. Prepare a 250mU/ μ l stock and add 2.5 μ l of stock per assay point.
- Magnesium/ATP Cocktail:** 50mM magnesium acetate, 0.5mM ATP.
- [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.)
- Modified Crosstide:** Use at a final assay concentration of 30 μ M. Prepare a 300 μ M stock and add 2.5 μ l of stock per assay point.

Assay Procedure:

Stage One: *Activation of Akt1/PKB α by MAPKAP-K2*

- Add 2.5 μ l of 10 x Akt1/PKB alpha activation buffer to a microcentrifuge tube.
- Add **2.5 μ l (5 μ g) of Akt1/PKB α , inactive.**
- Add 2.5 μ l (625mU) of **MAPKAP-K2, active.**
- Add 12.5 μ l of dH₂O.
- Add 5 μ l of magnesium/ATP cocktail.
- Incubate for 30 minutes at 30°C.

Stage Two: *Further activation of Akt1/PKB α by PDK1*

- Add 2.5 μ l (0.5 μ g) of **PDK1** to appropriate tubes
- Incubate for a further 30 minutes at 30°C.
- Stop reaction by diluting reaction 10–100 fold and incubating on ice.

Stage Three: *Phosphorylation of modified crosstide by Akt1 (96 well plate format).*

- Add 5 μ l of 5 x assay buffer to wells.
- Add **2.5 μ l of Stage Two** reaction mixture.
- Add 2.5 μ l of **modified crosstide.**
- Add 5 μ l of dH₂O.
- Add 10 μ l of diluted [γ -³³P]ATP mixture.
- Incubate for 10 minutes at 30°C.
- Stop the reaction by adding 5 μ l of 3% phosphoric acid.
- Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat.**
- Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
- Wash the filtermat once for 2 minutes with methanol.
- Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
- 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.

Certificate of Analysis

Akt1 Sequence Information

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

Recombinant Akt1 amino acid sequence:

```

1 MSFFHHHHHH DFDIPTTENL YFQGAMGSMS DVAIVKEGWL HKRGEYIKTW RPRYFLLKND
61 GTFIGYKERP QDVDQREAPL NNFSVAQCQL MKTERPRPNT FIIRCLQWTT VIERTFHVET
121 PEEREETTTA IQTVADGLKK QEEEEMDFRS GSPSDNSGAE EMEVSLAKPK HRVTMNEFEY
181 LKLLGKGTFTG KVILVKEKAT GRYYAMKILK KEVIVAKDEV AHTLTENRVL QNSRHPFLTA
241 LKYSFQTHDR LCFVMEYANG GELFFHLSRE RVFSEDRAF YGAEIVSALD YLHSEKNVVY
301 RDLKLENLML DKDGHKIDT FGLCKEIKD GATMKTFCTG PEYLAPVLE DNDYGRAVDW
361 WGLGVVMYEM MCGRLPFYNQ DHEKLFELIL MEEIRFPRTL GPEAKSLLSG LLKKDPKQRL
421 GGGSEDAKEI MQHRFFAGIV WQHVYEKKLS PPFKPQVTSE TDTRYFDEEF TAQMITITPP
481 DQDDSMCEVD SERRPHFPQF SYSASGTA

```

Recombinant Akt1 nucleotide sequence:

```

1 atgtcgttct tccatcacca tcaccatcac gatttcgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatggg atccatgagc gacgtggcta ttgtgaagga gggttggtcg
121 cacaacgag gggagtacat caagacctgg cggccacgct acttcctcct caagaatgat
181 ggcaccttca ttggctacaa ggagcggcgc caggatgtgg accaacgtga ggctcccttc
241 aacaacttct ctgtggcgca gtgccagctg atgaagacgg agcggccccg gcccacacc
301 ttcacatcatc gctgcctgca gtggaccact gtcacgaac gcaccttcca tgtggagact
361 cctgaggagc gggaggagtg gacaaccgcc atccagactg tggctgacgg cctcaagaag
421 caggaggagg aggagatgga cttccggctg ggctcaccca gtgacaactc aggggctgaa
481 gagatggagg tgtccctggc caagccaag accgcgtga ccatgaacga gtttgagtac
541 ctgaagctgc tgggcaaggg cacttcggc aaggtgatcc tgggtgaagga gaaggccaca
601 ggccgctact acgcatgaa gatcctcaag aaggaagtca tcgtggccaa ggacgaggtg
661 gcccacacac tcaccgagaa ccgcgtcctg cagaactcca ggcacccctt cctcacagcc
721 ctgaagtact cttccagac ccacgaccgc ctctgctttg tcatggagta cgccaacggg
781 ggcgagctgt tcttccacct gtcccgggag cgtgtgttct ccgaggaccg ggcccgttct
841 tatggcgctg agattgtgtc agccctggac tacctgcact cggagaagaa cgtggtgtac
901 cgggacctca agctggagaa cctcatgctg gacaaggacg ggcacattaa gatcacagac
961 ttcgggctgt gcaaggagg gatcaaggac ggtgccacca tgaagacctt ttgcggcaca
1021 cctgagtacc tggccccga ggtgctggag gacaatgact acggcgtgac agtggtactg
1081 tgggggctgg gcgtggcat gtacgagatg atgtgcggtc gcctgccctt ctacaaccag
1141 gaccatgaga agctttttga gctatcctc atggaggaga tccgcttccc gcgcacgctt
1201 ggtcccgagg ccaagtcctt gctttcaggg ctgctcaaga aggaccccaa gcagaggctt
1261 ggcgggggct ccgaggagc caaggagatc atgcagcatc gcttctttgc cggtatcgtg
1321 tggcagcacg tgtacgagaa gaagctcagc ccacccttca agccccaggt cacgtcggag
1381 actgacacca ggtattttga tgaggagttc acggcccaga tgatcaccat cacaccacct
1441 gaccaagatg acagcatgga gtgtgtggac agcagcgcga ggccccactt cccccagttc
1501 tcctactcgg ccagcgccac ggcctga

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.