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Certificate of Analysis

Akt1/PKBα, 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 alpha, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 67.7% by SDS-PAGE and Coomassie blue staining. MW = 59kDa.

Specific Activity (Parent lot# D8SN004U): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with PDK1 and MAPKAP-K2.

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 6 months 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.

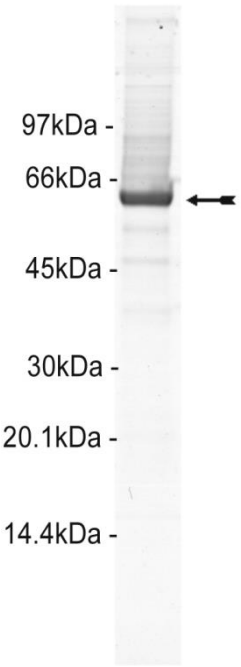
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: 0.2mg/ml unactive Akt1/PKBα 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.

Active PDK1	Active MAPKAP-K2	Unactive Akt1/PKBα	Mean cpm	Comments
None	None	5µg	5	Background
0.5µg	625mU	5µg	62529	Kinase activity

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 3µg of unactive Akt1/PKBα.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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Kinase Cascade Assay Protocol

Stock Solutions:

1. **Akt1/PKBα Activation Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 2mM NaCl, 1% 2-mercaptoethanol.
2. **5 x Assay Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
3. **Akt1/PKBα, unactive:** 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 2.5µl per assay point.
4. **PDK1, active (Catalogue#14-452):** Use at a final assay concentration of 0.02mg/ml. Prepare a 0.2mg/ml stock and add 2.5µl of stock per assay point.
5. **MAPKAP-K2, active (Catalogue# 14-337):** Use at a final assay concentration of 25U/ml. Prepare a 250mU/µl stock and add 2.5µl of stock per assay point.
6. **Magnesium/ATP Cocktail:** 50mM magnesium acetate, 0.5mM ATP.
7. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)
8. **Modified Crosstide:** Use at a final assay concentration of 30µM. Prepare a 300µM stock and add 2.5µl of stock per assay point.

Assay Procedure:

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

1. Add 2.5µl of 10 x Akt1/PKB alpha activation buffer to a microcentrifuge tube.
2. Add **2.5µl (5µg) of Akt1/PKBα, unactive**.
3. Add 2.5µl (625mU) of **MAPKAP-K2, active**.
4. Add 12.5µl of dH_2O.
5. Add 5µl of magnesium/ATP cocktail.
6. Incubate for 30 minutes at 30°C.

Stage Two: *Further activation of Akt1/PKBα by PDK1*

1. Add 2.5µl (0.5µg) of **PDK1** to appropriate tubes
2. Incubate for a further 30 minutes at 30°C.
3. Stop reaction by diluting reaction 10–100 fold and incubating on ice.

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

1. Add 5µl of 5 x assay buffer to wells.
2. Add **2.5µl** of **Stage Two** reaction mixture.
3. Add 2.5µl of **modified crosstide**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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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 PEEREEWTTA IQTVADGLKK QEEEEMDFRS GSPSDNSGAE EMEVSLAKPK HRVTMNEFEY
 181 LKLLGKGTFG KVILVKEKAT GRYYAMKILK KEVIVAKDEV AHTLTENRVL QNSRHPFLTA
 241 LKYSFQTHDR LCFVMEYANG GELFFHLSRE RVFSEDRARF YGAEIVSALD YLHSEKNVVY
 301 RDLKLENLML DKDGHIKITD FGLCKEGIKD GATMKTFCGT PEYLAPEVLE DNDYGRAVDW
 361 WGLGVVMYEM MCGRLPFYNQ DHEKLFELIL MEEIRFPRTL GPEAKSLLSG LLKKDPKQRL
 421 GGGSEDAKEI MQHRFFAGIV WQHVYEKKLS PPFKPQVTSE TDTRYFDEEF TAQMITITPP
 481 DQDDSMECVD SERRPHFPQF SYSASGTA
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Recombinant Akt1 nucleotide sequence:

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   1 atgtcgttct tccatcacca tcaccatcac gatttcgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg atccatgagc gacgtggcta ttgtgaagga gggttggctg
 121 cacaaacgag gggagtacat caagacctgg cggccacgct acttcctcct caagaatgat
 181 ggcaccttca ttggctacaa ggagcggccg caggatgtgg accaacgtga ggctcccctc
 241 aacaacttct ctgtggcgca gtgccagctg atgaagacgg agcggccccg gcccaacacc
 301 ttcatcatcc gctgcctgca gtggaccact gtcatcgaac gcaccttcca tgtggagact
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1501 tcctactcgg ccagcggcac ggcctga
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Reviewed and approved by site quality representative.