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Certificate of Analysis

Akt1/PKBα (S473D), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-453, 14-453-K, 14-453M
Parent Lot # 1600485

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 human Akt1/PKB alpha (S473D), residues 118–end containing the mutation S473D. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Activated using PDK1 (cat# 14-452) and repurified using heparin Sepharose. Purity 97.2% by SDS-PAGE and Coomassie blue staining. MW = 45kDa.

Specific Activity (Parent lot# 1600485): 6080U/mg, where one unit of Akt1/PKB alpha (S473D), active activity is defined as 1nmol phosphate incorporated into 30µM modified crosstide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.069mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

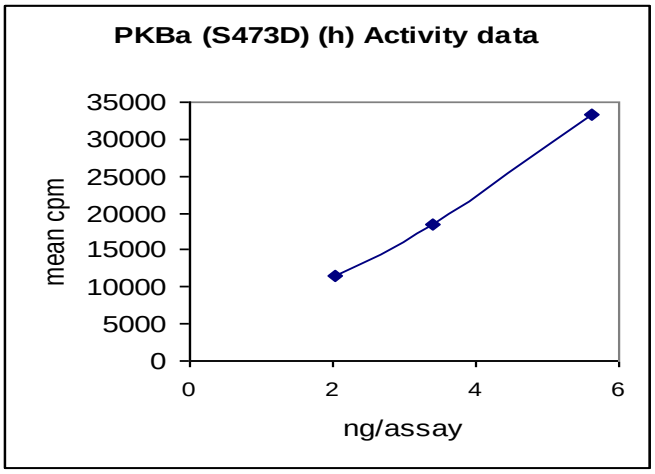
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

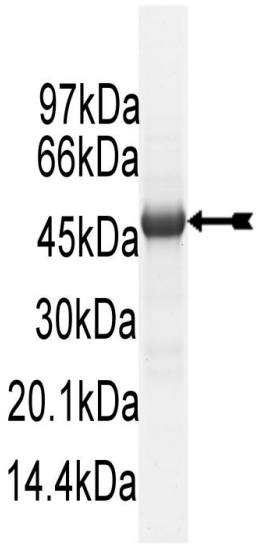
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 2.0–5.6ng of this lot of enzyme phosphorylated 30µM modified crosstide 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 product identity as Akt1/PKBα (S473D) with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Akt1/PKBα (S473D), active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Modified Crosstide:** Use at a final assay concentration of 30µM. Prepare a 300µM stock and add 2.5µl of stock per assay point.
3. **Akt1/PKBα (S473D), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethnaol, 1mg/ml BSA. Use 2.0–5.6ng per assay point.
4. **[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.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **modified crosstide**.
3. Add **2.5µl (2.0–5.6ng) Akt1/PKBα (S473D), active**.
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/PKB alpha (S473D) Sequence Information

Protein human Akt1 (118–end, S473D)

Tags *N*-terminal 6His

Native sequence M29 of the recombinant protein is equivalent to M118 of human Akt1

Accession number GenBank M63167. S478G with regard to this accession, reported in EMBL BE206796. S473D conflict mimics phosphorylation of S473.

Recombinant Akt1 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMGSMD FRSGSPSDNS GAEEMEVSLA KPKHRVTMNE
  61 FEYLKLLGKG TFGKVILVKE KATGRYYAMK ILKKEVIVAK DEVAHTLTEN RVLQNSRHPF
 121 LTALKYSFQT HDRLCFVMEY ANGGELFFHL SRERVFSEDR ARFYGAEIVS ALDYLHSEKN
 181 VVYRDLKLEN LMLDKDGHIK ITDFGLCKEG IKDGATMKTF CGTPEYLAPE VLEDNDYGRA
 241 VDWWGLGVVM YEMMCGRLPF YNQDHEKLFE LILMEEIRFP RTLGPEAKSL LSGLLKKDPK
 301 QRLGGGSEDA KEIMQHRFFA GIVWQHVYEK KLSPPFKPQV TSETDTRYFD EEFTAQMITI
 361 TPPDQDDSME CVDSERRPHF PQFDYSASGT A
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Recombinant Akt1 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg atccatggac ttccggtcgg gctcacccag tgacaactca
 121 ggggctgaag agatggaggt gtccctggcc aagcccaagc accgcgtgac catgaacgag
 181 tttgagtacc tgaagctgct gggcaagggc actttcggca aggtgatcct ggtgaaggag
 241 aaggccacag gccgctacta cgccatgaag atcctcaaga aggaagtcat cgtggccaag
 301 gacgaggtgg cccacacact caccgagaac cgcgtcctgc agaactccag gcacccttc
 361 ctcacagccc tgaagtactc tttccagacc cacgaccgcc tctgctttgt catggagtac
 421 gccaacgggg gcgagctgtt cttccacctg tcccgggagc gtgtgttctc cgaggaccgg
 481 gcccgcttct atggcgctga gattgtgtca gccctggact acctgcactc ggagaagaac
 541 gtggtgtacc gggacctcaa gctggagaac ctcatgctgg acaaggacgg gcacattaag
 601 atcacagact tcgggctgtg caaggagggg atcaaggacg gtgccaccat gaagaccttt
 661 tgcggcacac ctgagtacct ggcccccgag gtgctggagg acaatgacta cggccgtgca
 721 gtggactggt gggggctggg cgtggtcatg tacgagatga tgtgcggtcg cctgcccttc
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1021 acgtcggaga ctgacaccag gtattttgat gaggagttca cggcccagat gatcaccatc
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1141 ccccagttcg actactcggc cagcggcacg gcctga
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Reviewed and approved by site quality representative.