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Certificate of Analysis

ACK1, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-756, 14-756-K, 14-756M
Parent Lot # D7EN072U

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 GST-tagged recombinant, human ACK1, residues 1–389. Expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 65% by SDS-PAGE and Coomassie blue staining. MW = 71.5kDa.

Specific Activity (Parent lot# D7EN072U): 249U/mg, where one unit of ACK1, active activity is defined as 1nmol phosphate incorporated into 400µM (EFPIYDFLPAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.822mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

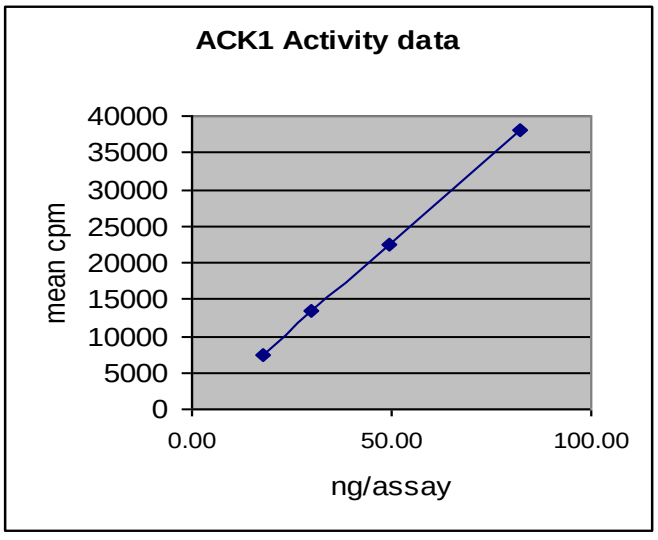
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

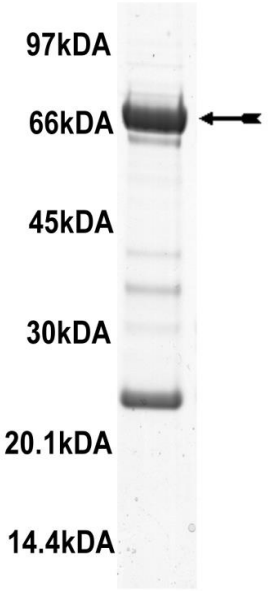
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 18–82ng of this lot of enzyme phosphorylated 400µM (EFPIYDFLPAKKK) 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 ACK1 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 ACK1, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(EFPIYDFLPAKKK):** Use at a final assay concentration of 400µM. Prepare a 4mM stock and add 2.5µl of stock per assay point.
3. **ACK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 18–82ng 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 **(EFPIYDFLPAKKK)**.
3. Add **2.5µl (18–82ng) ACK1, 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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ACK1 Sequence Information

Protein	human ACK1
Tags	*N*-terminal GST
Native sequence	M231 of recombinant sequence is equivalent to M1 of the native ACK1
Accession number	GenBank NM_005781

Recombinant ACK1 amino acid sequence:

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   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF MQPEEGTGWL
 241 LELLSEVQLQ QYFLRLRDDL NVTRLSHFEY VKNEDLEKIG MGRPGQRRLW EAVKRRKALC
 301 KRKSWMSKVF SGKRLEAEFP PHHSQSTFRK TSPAPGGPAG EGPLQSLTCL IGEKDLRLLE
 361 KLGDGSFGVV RRGEWDAPSG KTVSVAVKCL KPDVLSQPEA MDDFIREVNA MHSLDHRNLI
 421 RLYGVVLTPP MKMVTELAPL GSLLDRLRKH QGHFLLGTLS RYAVQVAEGM GYLESKRFIH
 481 RDLAARNLLL ATRDLVKIGD FGLMRALPQN DDHYVMQEHR KVPFAWCAPE SLKTRTFSHA
 541 SDTWMFGVTL WEMFTYGQEP WIGLNGSQIL HKIDKEGERL PRPEDCPQDI YNVMVQCWAH
 601 KPEDRPTFVA LRDFLLEAQ
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Recombinant ACK1 nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
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 781 aacgtcaccc gcctgtccca ctttgagtac gtcaagaatg aggacctgga gaagatcggc
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1081 aagctgggtg atggttcctt tggcgtggtg cgcaggggcg agtgggacgc gccctcaggg
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1261 cgcctctacg gggtggtgct cacgccgccc atgaagatgg tgacagagct ggcacctctg
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1741 ccccggcccg aggactgtcc ccaggacatc tacaacgtca tggtccagtg ctgggctcac
1801 aagccagagg acagacccac gtttgtggcc ctgcgggact tcctgctgga ggcccagtaa
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Reviewed and approved by site quality representative.