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Certificate of Analysis

DAPK1, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-692, 14-692-K, 14-692M
Parent Lot # 33232U

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 DAPK1, amino acids 1–296, expressed by baculovirus in Sf21 insect cells. Purified using glutathione-agarose. Purity 97% by SDS-PAGE and Coomassie blue staining. MW = 60.9kDa.

Specific Activity (Parent lot# 33232U): 879U/mg, where one unit of DAPK1 activity is defined as 1nmol phosphate incorporated into 250µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.3mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

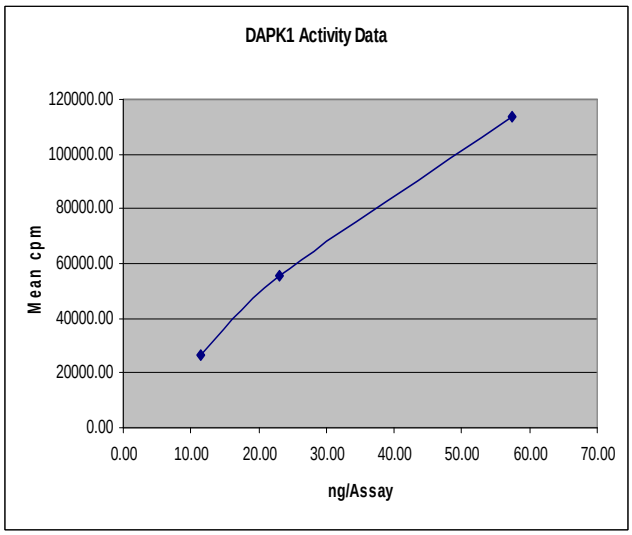
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

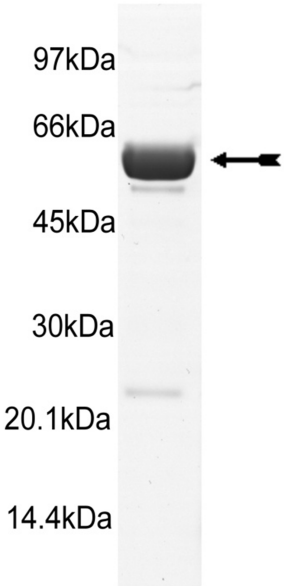
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 11.5–57.5ng of this lot of enzyme phosphorylated 250µM (KKLNRTLSFAEPG) 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 DAPK1 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 DAPK1, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(KKLNRTLSFAEPG):** Use at a final concentration of 250µM. Make up a 2.5mM stock. Use 2.5µl of stock per assay point.
3. **DAPK1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 11.5–57.5ng 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 **(KKLNRTLSFAEPG)**.
3. Add **2.5µl (11.5–57.5ng) DAPK1, 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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DAPK1 Sequence Information

Protein Human DAPK1

Tags *N*-terminal GST

Native sequence M231 of recombinant protein is equivalent to M1 of human DAPK1

Accession number GenBank NM_004938

Recombinant DAPK1 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 MTVFRQENVD
241 DYYDTGEELG SGQFAVVKKC REKSTGLQYA AKFIKKRRTK SSRRGVSRED IEREVSILKE
301 IQHPNVITLH EVYENKTDVI LILELVAGGE LFDFLAEKES LTEEEATEFL KQILNGVYYL
361 HSLQIAHFDL KPENIMLLDR NVPKPRIKII DFGLAHKIDF GNEFKNIFGT PEFVAPEIVN
421 YEPLGLEADM WSIGVITYIL LSGASPFLGD TKQETLANVS AVNYEFEDEY FSNTSALAKD
481 FIRRLLVKDP KKRMTIQDSL QHPWIKPKDT QQALSRKASA VNMEKF
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Recombinant DAPK1 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
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
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1201 ggaaatgaat ttaaaaacat atttgggact ccagagtttg tcgctcctga gatagtcaac
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1381 gctgtcaact acgaatttga ggatgaatac ttcagtaata ccagtgccct agccaaagat
1441 ttcataagaa gacttctggt caaggatcca aagaagagaa tgacaattca agatagtttg
1501 cagcatccct ggatcaagcc taaagataca caacaggcac ttagtagaaa agcatcagca
1561 gtaaacatgg agaaattcta a
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