

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, active activity is defined as 1nmol phosphate incorporated into 250μM (KKLNRTLSEFAEPG) 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.

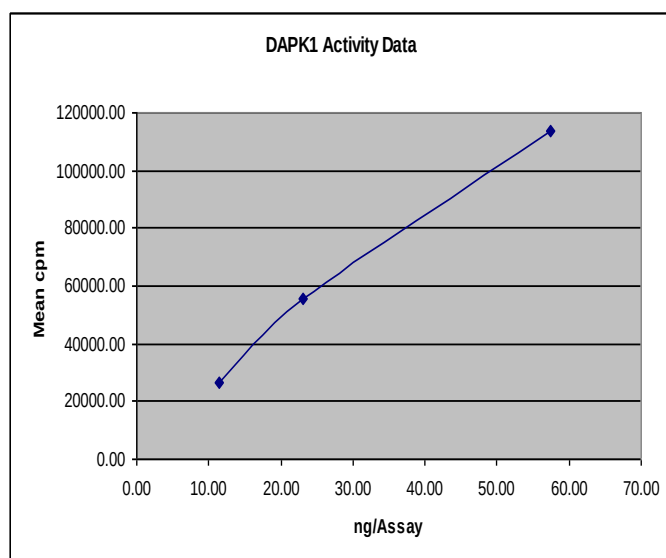
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

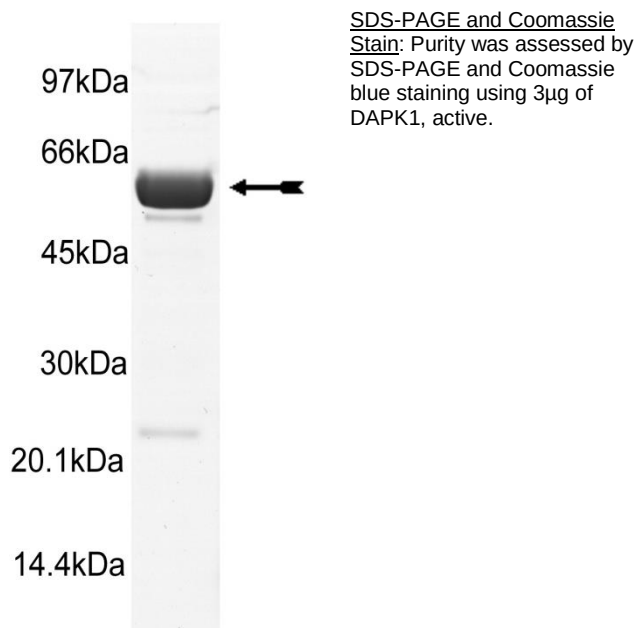
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 11.5–57.5ng of this lot of enzyme phosphorylated 250μM (KKLNRTLSEFAEPG) 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.



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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 -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.)

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 dH₂O.
5. Add 10µl of diluted [γ -³³P]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  KGLVQPTRL  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 DYYDTGEEEL  SGQFAVVKKC  REKSTGLQYA  AKFIKKRRTK  SSRGVSRRED  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  tactagggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
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181  ggtgatgtta  aattaacaca  gtctatggcc  atcatagctt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
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1561 gtaaaccatg  agaaattcta  a

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