

Certificate of Analysis

HPK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-968, 14-968-K, 14-968M

Parent Lot # D15MP003N

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 and C-terminal 6His-tagged, recombinant, human HPK1, amino acids 1-346 expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 81% by SDS-PAGE and Coomassie blue staining. MW = 67kDa.

Specific Activity (Parent lot# D15MP003N): 1864U/mg, where one unit of HPK1, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.41mg/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.

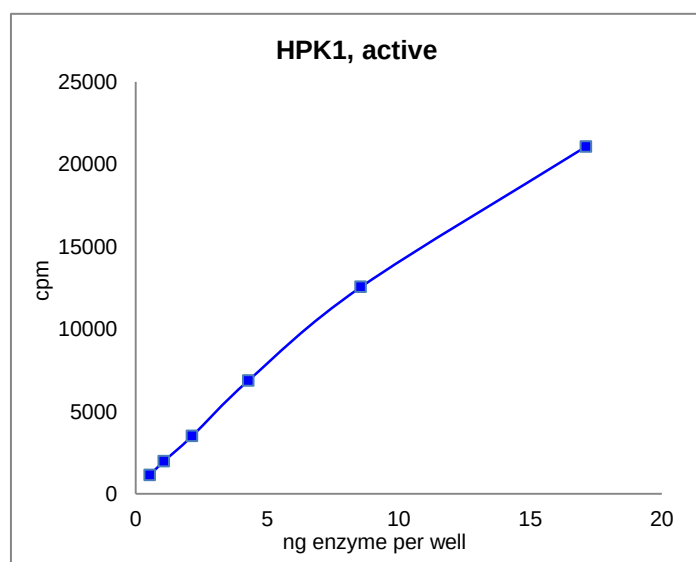
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

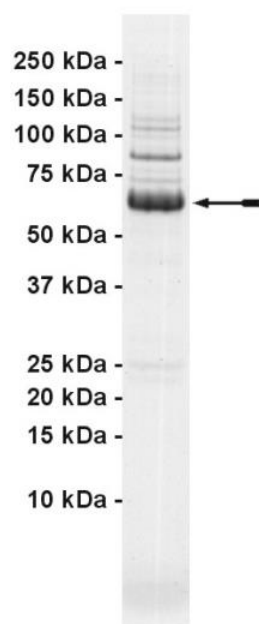
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1–17ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein 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 HPK1 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 HPK1, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Myelin basic protein:** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
- 3. HPK1, active** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–17ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[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 myelin basic protein.
3. Add 5µl of dH₂O.
4. Add **2.5µl (1–17ng) HPK1, active.**
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 dried 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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HPK1, active Sequence Information

Protein	Human HPK1
Tags	N-terminal GST and C-terminal 6His
Native sequence	M230 of the recombinant protein is equivalent to M1 of human HPK1
Accession number	GenBank NM_007181.5

Recombinant HPK1 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 KRIEAIQID   KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSKELM  DVVDPDIFNR
241 DPRDHYDLLQ  RLGGGTYGVE  FKARDKVS GD  LVALKMKVME  PDDDVSTLQK  EILILKTCRH
301 ANIVAYHGSY  LWLQKLWICM  EFCGAGSLQD  IYQVTGSLSE  LQISYVCREV  LQGLAYLHSQ
361 KKIHRDIKGA  NILINDAGEV  RLADFGISAQ  IGATLARRLS  FIGTPYWMAF  EVAVALKGG
421 YNELCDIWSL  GITAIELAE  QPPLFDVHPL  RVLFLMTKSG  YQPPRLKEKG  KWSAAFHNFI
481 KVTLTSPKPK  RPSATKMLSH  QLVSQPGLNR  GLILDLLDKL  KNPGKGPSIG  DIEDEEPELP
541 PAIPRRIRST  HRSSSLGIPD  ADCCRRHMEF  RKLRGPGPG  HHHHHH

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Recombinant HPK1 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
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121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcccta  ttatatattgat
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1621  cctgctatcc  ctcgcggtg  cagatccacc  caccgctcca  gctctctggg  gatcccagat
1681  gcagactgct  gtcggcgga  catggagtcc  aggaagctcc  gaggaggccc  gggccctggc

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1741 catcaccatc accatcacta a

Reviewed and approved by site quality representative.

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