

Certificate of Analysis

HIPK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-679, 14-679-K, 14-679M

Parent Lot # D9BN005U

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 HIPK1, amino acids 158–555, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Purity 96.5% by SDS-PAGE and Coomassie blue staining. MW = 49.6kDa.

Specific Activity (Parent lot# D9BN005U): 1277U/mg, where one unit of HIPK1, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.679mg/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.

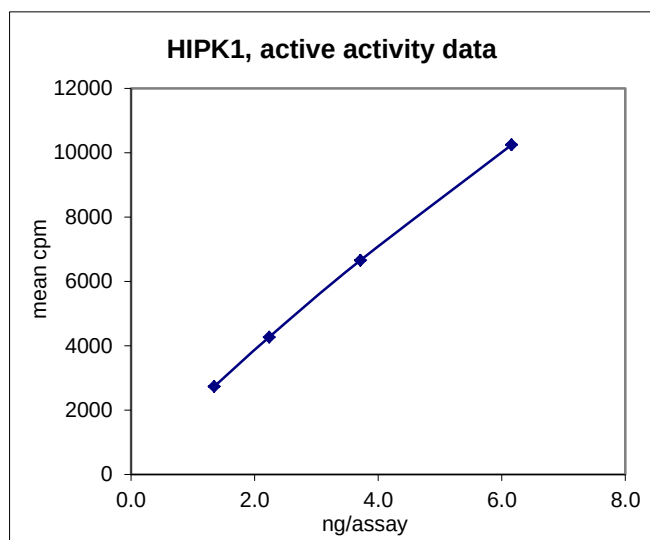
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

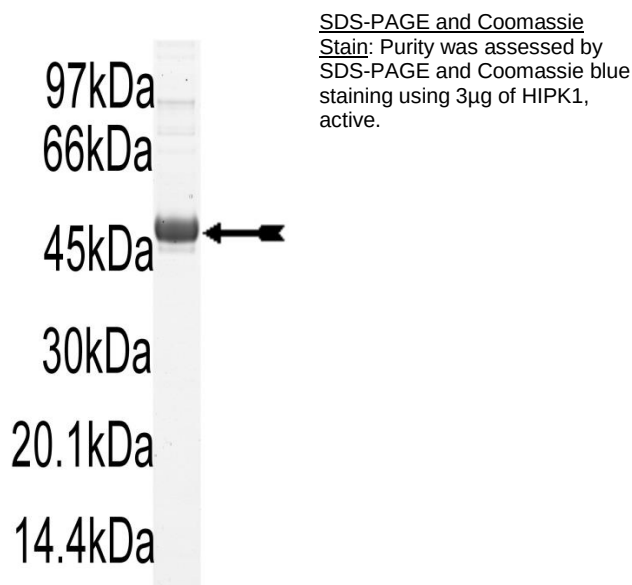
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.3–6.2ng of this lot of enzyme phosphorylated 0.33mg/ml MBP 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 HIPK1 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. **Myelin Basic Protein (MBP):** Use at a final concentration of 0.33mg/ml. Make a 3.3mg/ml stock. Add 2.5µl of stock per assay point.
3. **HIPK1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.3–6.2ng 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 0.33mg/ml **MBP**.
3. Add **2.5µl (1.3–6.2ng) HIPK1, 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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HIPK1 Sequence Information

Protein	human HIPK1
Tags	N-terminal 6His
Native sequence	T31 of recombinant protein is equivalent to T158 of native human HIPK1
Accession number	GenBank NM_152696

Recombinant HIPK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF TTTTVTTKSS SSSGEGDYQL VQHEILCSMT
61 NSYEVLEFLG RGTFGQVAKC WKRSTKEIVA IKILKNHPSY ARQGQIEVSI LSRLSSENAD
121 EYNFVRSYEC FQHKNHTCLV FEMLEQNLVD FLKQNKFSPL PLKYIRPILQ QVATALMKLK
181 SLGLIHADLK PENIMLVDPV RQPYRVKVID FGSASHVSKA VCSTYLQSRV YRAPEIILGL
241 PFCEAIDMWS LGCVIAELFL GWPLYPGASE YDQIRYISQT QGLPAEYLLS AGTKTTRFFN
301 RDPNLGYPLW RLKTPPEHEL ETGIKSKEAR KYIFNCLDDM AQVNMSTDLE GTDMLAEKAD
361 RREYIDLLKK MLTIDADKRI TPLKTLNHQF VTMTLLDFFP HSNHVKSCFQ NMEICKRRVH
421 MYDTSVSI

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Recombinant HIPK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc acaaccacca ctgtgaccac aaagagtagc
121 agttccagcg gagaagggga ttaccagctg gtccagcatg agatcctttg ctctatgacc
181 aatagctatg aagtcttgga gttcctaggc cgggggacat ttggacaggt ggctaagtgc
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301 gccagacaag gacagattga agtgagcatc ctttcccgcc taagcagtga aaatgctgat
361 gagtataatt ttgtccgttc atacgagtgc tttcagcata agaatacacac ctgccttggt
421 tttgaaatgt tggagcagaa cttatatgat tttctaaagc aaaacaaatt tagccactg
481 ccactcaagt acatcagacc aatcttgtag cagggtggca cagccttgat gaagctcaag
541 agtcttggtc tgatccacgc tgaccttaag cctgaaaaca tcatgctggt tgatccagtt
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1201 catagcaatc atgttaagtc ttgttttcag aacatggaga tctgcaagcg gagggttcac
1261 atgtatgata cagtgagtca gatctaa

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