

Certificate of Analysis

HIPK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-623, 14-623-K, 14-623M

Parent Lot # 25713U

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 HIPK2, amino acids 165–564, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 49.6kDa.

Specific Activity (Parent lot# 25713U): 2066U/mg, where one unit of HIPK2, 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: 2.11mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

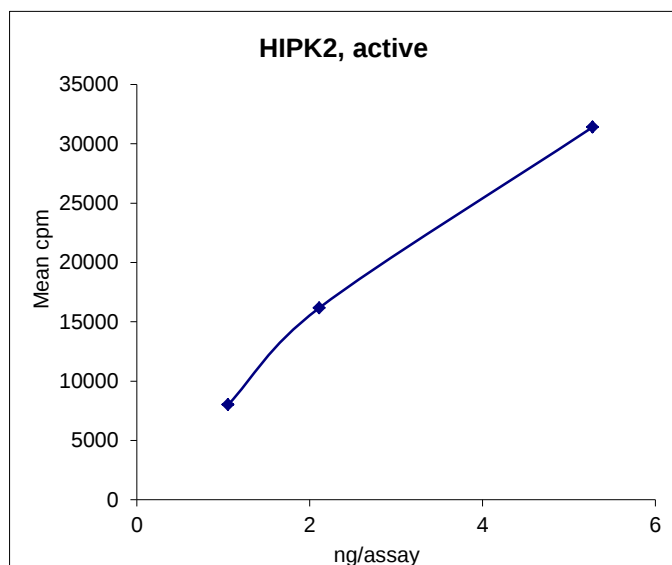
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

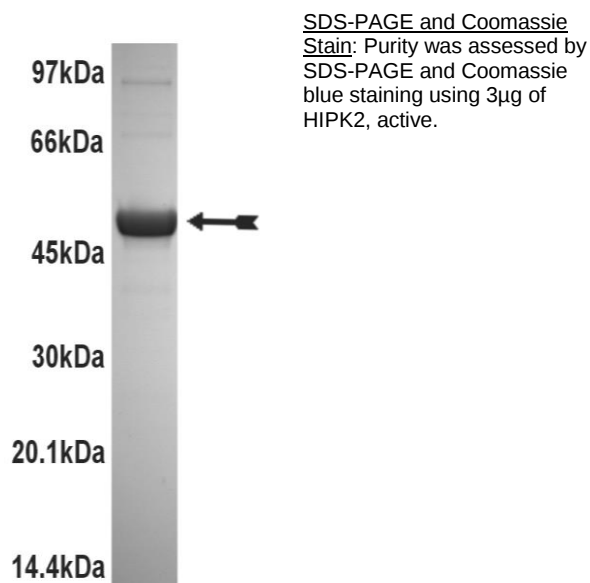
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1–5ng of this lot of enzyme phosphorylated 0.33 mg/ml myelin basic protein (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 HIPK2 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 assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
3. **HIPK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–5ng 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 2.5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **myelin basic protein (MBP)**.
3. Add **2.5µl (1–5ng) HIPK2, 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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HIPK2 Sequence Information

<u>Protein</u>	human HIPK2
<u>Tags</u>	N-Terminal 6His
<u>Native sequence</u>	T31 of recombinant sequence is equivalent to T165 of native human HIPK2
<u>Accession number</u>	GenBank AF326592. The recombinant protein contains the amino acid substitution I471N (native coordinates) with respect to GenBank AF326592. This conflict is reported in GenBank accession nos. AF207702, AF208291, NM022740.

Recombinant HIPK2 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF TATTSTATSK NSGSNSEGDY QLVQHEVLCS
61 MTNTYEVLEF LGRGTFGQVV KCWKRGTEI VAIKILKNHP SYARQGQIEV SILARLSTES
121 ADDYNFVRAY ECFQHKNHTC LVFEMLEQNL YDFLKQNKFS PLPLKYIRPV LQQVATALMK
181 LKSLGLIHAD LKPENIMLVD PSRQPYRVKV IDFGSASHVS KAVCSTYLQS RYYRAPEIIL
241 GLPFCEAIDM WSLGCVIAEL FLGWPLYPGA SEYDQIRYIS QTQGLPAEYL LSAGTKTTRF
301 FNRDTSPPYP LWRLKTPDDH EAETGIKSKE ARKYIFNCLD DMAQVNMTTD LEGSDMLVEK
361 ADRREFIDLL KKMLTIDADK RITPIETLNH PFVTMTHLLD FPHSTHVKSC FQNMEICKRR
421 VNMYDTVNQS

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Recombinant HIPK2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc actgccacca cgtctactgc cacctccaaa
121 aacagcggct ccaacagcga gggcgactat cagctgggtgc agcatgaggt gctgtgctcc
181 atgaccaaca cctacgaggt cttagagtgc ttgggcccag ggacgtttgg gcaagtgggc
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301 tcctatgccc gacaaggtca gattgaagtg agcatcctgg cccggttgag cacggagagt
361 gccgatgact ataacttcgt ccgggcctac gaatgcttcc agcacaagaa ccacacgtgc
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1201 tttccccaca gcacacacgt caaatcatgt ttccagaaca tggagatctg caagcgtcgg
1261 gtgaatatgt atgacacggt gaaccagagc taa

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