

Certificate of Analysis

HIPK4, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-003, 15-003-K, 15-003M

Parent Lot # 223239

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: C-terminal 6His tagged, recombinant, human HIPK4 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 71% by SDS-PAGE and Coomassie blue staining. MW = 71 kDa.

Specific Activity (Parent lot# 223239): 858 U/mg, where one unit of HIPK4 activity is defined as 1 nmol phosphate incorporated into 250 μ M RRRFRPASPLRGPPK per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.53 mg/ml of enzyme in 50 mM Tris/HCl pH7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

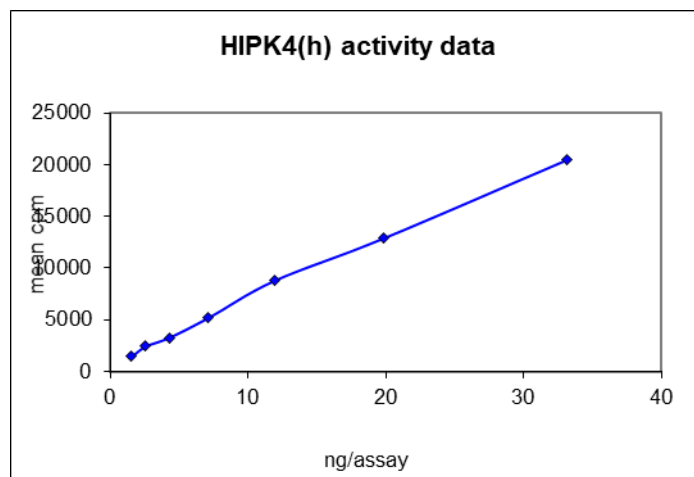
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

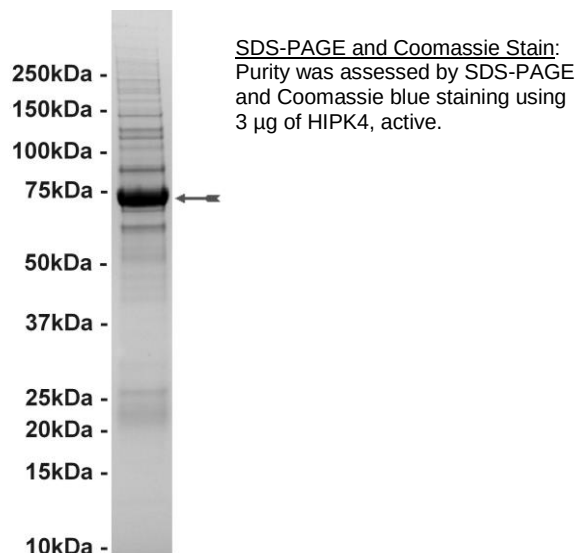
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1.6–33.1 ng of this lot of enzyme phosphorylated 250 μ M RRRFRPASPLRGPPK 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 HIPK4 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH7.0, 1 mM EDTA.
- 2. RRRFRPASPLRGPPK:** Use at a final assay concentration of 250 μ M. Prepare a 2.5 mM stock and add 2.5 μ l of stock per assay point.
- 3. HIPK4, active:** Dilute with 20 mM MOPS/NaOH pH7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 1.6–33.1 ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of RRRFRPASPLRGPPK.
3. Add **2.5 μ l (1.6–33.1 ng) HIPK4, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the dried filtermat to a sealable plastic bag and add 4 ml 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 1 μ l of 30% phosphoric acid.

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HIPK4, active Sequence Information

<u>Protein</u>	Human HIPK4
<u>Tags</u>	C-terminal 6His
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human HIPK4
<u>Accession number</u>	GenBank BC034501.2

Recombinant HIPK4 amino acid sequence:

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1  MSTIQSETDC YDIIEVLGKG TFGEVAKGWR RSTGEMVAIK ILKNDAYRNR IIKNELKLLH
61  CMRGLDPEEA HVIRFLEFFH DALKFYLVFE LLEQNLFEFQ KENNFAPLPA RHIRTVTLQV
121 LTALARLKEL AIIHADLKPE NIMLVDQTRC PFRVKVIDFG SASIFSEVRY VKEPYIQSRF
181 YRAPEILLGL PFCEKVDVWS LGCVMAELHL GWPLYPGNNE YDQVRYICET QGLPKPHLLH
241 AACKAHFFFK RNPHPDAANP WQLKSSADYL AETKVRPLER RKYMLKSLDQ IETVNGGSVA
301 SRLTFPDREA LAEHADLKSM VELIKRMLTW ESHERISPSA ALRHPFVSMQ QLRSAHETTH
361 YYQLSLRSYR LSLQVEGKPP TPVVAEDGT PYYCLAEKEE AAGMGSVAGS SPFFREEKAP
421 GMQRAIDQLD DLSLQEAGHG LWGETCTNAV SDMMVPLKAA ITGHHVPDSG PEPILAFYSS
481 RLAGRHKARK PPAGSKSDSN FSNLIRLSQV SPEDDRPCRG SSWEEGEHLG ASAEPLAILQ
541 RDEDGPNIDN MTMEAERPDP ELFDPSSCP G EWLSEPDCTL ESVRGPRAGQ LPPRRSHQHG
601 PPRGATSFLQ HVTGHHGPGP GHHHHHH

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Recombinant HIPK4 nucleotide sequence:

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1  atgtccacca tccagtcgga gactgactgc tacgacatca tgcaggtcctt gggcaagggg
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121 atcctcaaga atgacgccta ccgcaaccgc atcatcaaaa acgagctgaa gctgctgcac
181 tgcattgcag gcctagaccc tgaagaggcc cacgtcatcc gcttccttga gttcttccat
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1381 atcactggcc accatgtgcc cgactcgggc cctgagccca tcttggcctt ctacagcagc
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1621 cgagatgagg atgggcccac cattgacaac atgaccatgg aagctgagag gccagaccct
1681 gagctcttcg accccagcag ctgtcctgga gaatggctga gtgagccaga ctgcaccctg
1741 gagagcgtca ggggcccacg ggctcagggg ctcccacccc gccgctcca ccagcatggt
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1861 ggccatcacc atcaccatca ctaa
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