

Certificate of Analysis

HIPK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-680, 14-680-K, 14-680M

Parent Lot # 2189233

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 HIPK3, amino acids 161–562, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Purity 93.3% by SDS-PAGE and Coomassie blue staining. MW = 49.7kDa.

Specific Activity (Parent lot# 2189233): 747U/mg, where one unit of HIPK3, active activity is defined as 1nmol phosphate incorporated into 1mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.65mg/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.

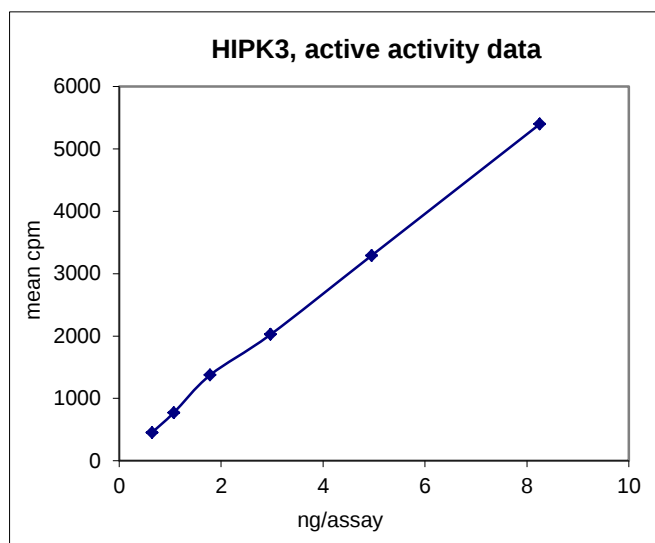
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

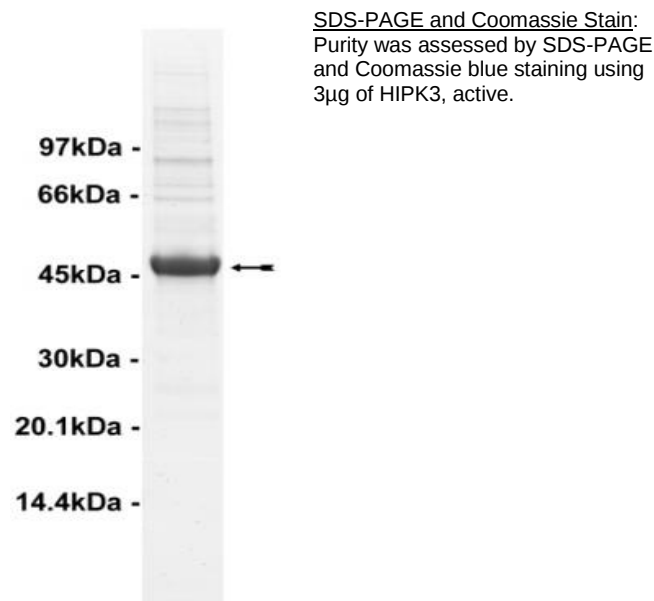
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.6–8.3ng of this lot of enzyme phosphorylated 1mg/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 HIPK3 with the translated native sequence listed on page three.



Certificate of Analysis

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 concentration of 1mg/ml. Make a 10mg/ml stock. Add 2.5µl of stock per assay point.
3. **HIPK3, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.6–8.3ng 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 1mg/ml **MBP**.
3. Add **2.5µl (0.6–8.3ng) HIPK3, 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.

Certificate of Analysis

HIPK3 Sequence Information

<u>Protein</u>	Human HIPK3
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	P31 of recombinant protein is equivalent to P161 of human HIPK3
<u>Accession number</u>	GenBank NM_005734

Recombinant HIPK3 amino acid sequence:

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF PVTVTATTG SKQNCTTGEG DYQLVQHEVL
61 CSMKNTYEVL DFLGRGTFGQ VVKCWKRGTN EIVAIIKILKN HPSYARQGQI EVSILARLST
121 ENADEYNFVR AYECFQHRNH TCLVFEMLEQ NLYDFLKQNK FSPLPLKVIR PILQQVATAL
181 KKLKSLGLIH ADLKPENIML VDPVRQPYRV KVIDFGSASH VSKTVCSTYL QSRYYRAPEI
241 ILGLPFCEAI DMWSLGCVIA ELFLGWPLYP GALEYDQIRY ISQTQGLPGE QLLNVGKST
301 RFFCKETDMS HSGWRLKTLE EHEAETGMKS KEARKYIFNS LDDVAHVNTV MDLEGSDDLLA
361 EKADRREFVS LLKKMLLIDA DLRITPAETL NHPFVNMKHL LDFPHSNHVK SCFHIMDICK
421 SHLNSCDTNN HN

```

Recombinant HIPK3 nucleotide sequence:

```

1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc ccagtgcagc ttgtgacagc taccacagga
121 tcaaaacaga attgtaccac tggagaaggt gactatcagt tagtacagca tgaagtctta
181 tgctccatga aaaatactta cgaagtcctt gattttcttg gtcgaggcac gtttgccag
241 gtagttaaat gctggaaaag agggacaaat gaaattgtag caatcaaaat tttgaagaat
301 catccttctt atgccgtca aggtcaaata gaagtgcagc tattagcaag gctcagtact
361 gaaaatgctg atgaatataa ctttgtacga gcttatgaat gctttcagca ccgtaacct
421 acttgtttag tctttgagat gctggaacaa aacttgtagt actttctgaa acaaaataaa
481 tttagtcccc tgccactaaa agtgattcgg ccattcttc aacaagtggc cactgcactg
541 aaaaaaattga aaagtcttgg tttaattcat gctgatctca agccagagaa tattatgttg
601 gtggatcctg ttcggcagcc ttacagggtt aaagtaatag actttgggtc ggccagtcac
661 gtatcaaaga ctgtttgttc aacatatcta caatctcggc actacagagc tccagagatt
721 atattggggt tgccattttg tgaagccata gacatgtggt cattgggatg tgtgattgca
781 gaattatttc ttggatggcc gctctaccca ggagccttgg agtatgatca gattcgatac
841 atttctcaga ctcaagggtt gccaggagaa cagttgttaa atgtgggtac taaatccaca
901 agattttttt gcaaagaaac agatatgtct cattctggtt ggagattaaa gacattggaa
961 gagcatgagg cagagacagg aatgaagtct aaagaagcca gaaaatacat tttcaacagt
1021 ctggatgatg tagcgcatgt gaacacagtg atggatttgg aaggaagtga tcttttggct
1081 gagaaagctg atagaagaga atttgttagt ctgttgaaga aaatgttgct gattgatgca
1141 gatttaagaa ttactccagc tgagaccctg aaccatcctt ttgttaatat gaaacatctt
1201 ctagatttcc ctcatagcaa ccatgtaaag tcctgttttc atattatgga tatttgtaag
1261 tcccacctaa attcatgtga cacaataat cacaactga

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services