

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active HIPK3 [161 – 562]

Enzyme description:- HIPK3 [161 - 562]

Clone number:- DU 5525

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic 72, 983.07 daltons

Average Mass 73, 030.34 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.36

Purity:- 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

Substrate:-

Myelin basic protein Final concentration: 0.3 mg/ml

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Clone Data Sheet

HIPK3 [161 - 562]

Protein HIPK3 [161 - 562]

Clone number DU 5525

Species Human

Accession number NM_005734

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFGPLGSPEFPVTVVTTATTGS
KQNCTTGEGDYQLVQHEVLCSMKNTYEVLDFLGRGTFGQVVKCWRGTN
EIVAIKILKNHPSYARQGQIEVSILARLSTENADEYNFVRAYECFQHRN
HTCLVFEMLEONLYDFLKQNKFSPLPLKVIRPILOQVATALKKLKSGL
IHADLKPENIMLVDPVRQPYRVKVIDFGSASHVSKTVCSTYLQSRYYRA
PEIILGLPFCEAIDMWSLGCVIAELFLGWPLYPGALEYDQIRYISQTQG
LPGEQLLNVGTKSTRFFCKETDMSHSGWRLKTLEEHEAETGMKSKEARK
YIFNSLDDVAHVNTVMDLEGSDLLAEKADRREFVSLKMLLIDADLRI
TPAETLNHPFVNMKHLLD FPHSNHVKSCFHIMDICKSHLNSCDTNNHN

Native sequence Amino acids P161 – N562 of human HIPK3.
[Full length protein ends at residue Y1214]

Residue P235 of the fusion protein is equivalent to P161 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Eco*R1 sites of pGEX 6P-1

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Nucleotide
Sequence of insert

gaattcCCAGTGACAGTTGTGACAGCTACCACAGGATCAAAACAGAATT
GTACCACTGGAGAAGGTGACTATCAGTTAGTACAGCATGAAGTCTTATG
CTCCATGAAAAATACTTACGAAGTCCTTGATTTTCTTGGTGAGGCACG
TTTGGCCAGGTAGTTAAATGCTGGAAAAGAGGGACAAATGAAATTGTAG
CAATCAAAATTTTGAAGAATCATCCTTCTTATGCCCCGTCAAGGTCAAAT
AGAAGTGAGCATATTAGCAAGGCTCAGTACTGAAAATGCTGATGAATAT
AACTTTGTACGAGCTTATGAATGCTTTCAGCACCGTAACCATACTTGT
TAGTCTTTGAGATGCTGGAACAAAACCTTGTATGACTTTCTGAAACAAAA
TAAATTTAGTCCCCTGCCACTAAAAGTGATTTCGGCCATTCTTCAACAA
GTGGCCACTGCACTGAAAAAATTGAAAAGTCTTGGTTTAATTCATGCTG
ATCTCAAGCCAGAGAATATTATGTTGGTGGATCCTGTTTCGGCAGCCTTA
CAGGGTTAAAGTAATAGACTTTGGGTTCGGCCAGTCATGTATCAAAGACT
GTTTGTTCACATATCTACAATCTCGGTACTACAGAGCTCCAGAGATTA
TATTGGGGTTGCCATTTTGTGAAGCCATAGACATGTGGTCATTGGGATG
TGTGATTGCAGAATTATTTCTTGGATGGCCGCTCTACCCAGGAGCCTTG
GAGTATGATCAGATTCGATACATTTCTCAGACTCAAGGTTTGCCAGGAG
AACAGTTGTTAAATGTGGGTACTAAATCCACAAGATTTTTTTTGCAAAGA
AACAGATATGTCTCATTCTGGTTGGAGATTAAAGACATTGGAAGAGCAT
GAGGCAGAGACAGGAATGAAGTCTAAAGAAGCCAGAAAATACATTTTCA
ACAGTCTGGATGATGTAGCGCATGTGAACACAGTGATGGATTTGGAAGG
AAGTGATCTTTTGGCTGAGAAAGCTGATAGAAGAGAATTTGTTAGTCTG
TTGAAGAAAATGTTGCTGATTGATGCAGATTTAAGAATTACTCCAGCTG
AGACCCTGAACCATCCTTTTGTAAATATGAAACATCTTCTAGATTTCCC
TCATAGCAACCATGTAAAGTCCTGTTTTTCATATTATGGATATTTGTAAG
TCCCACCTAAATTCATGTGACACAAATAATCACAACtgagaattc