Division of Signal Tranduction 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:- Standard filter binding assay

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

Specific activity range:- To be determined

Division of Signal Tranduction Therapy

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	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**PVTVVTATTGS** **KQNCTTGEGDYQLVQHEVLCSMKNTYEVLDFLGRGTFGQVVKCWKRGTN** **EIVAIKILKNHPSYARQGQIEVSILARLSTENADEYNFVRAYECFQHRN** **HTCLVFEMLEQNLYDFLKQNKFSPLPLKVIRPILQQVATALKKLKSLGL** **IHADLKPENIMLVDPVRQPYRVKVIDFGSASHVSKTVCSTYLQSRYYRA** **PEIILGLPFCEAIDMWSLGCVIAELFLGWPLYPGALEYDQIRYISQTQG** **LPGEQLLNVGTKSTRFFCKETDMSHSGWRLKTLEEHEAETGMKSKEARK** **YIFNSLDDVAHVNTVMDLEGSDLLAEKADRREFVSLLKKMLLIDADLRI** **TPAETLNHPFVNMKHLLDFPHSNHVKSCFHIMDICKSHLNSCDTNNHN**
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 (LEVLFQGPL) residues 221 - 229
Cloning sites	*Eco*R1 sites of pGEX 6P-1

Nucleotide Sequence of insert

gaattcCCAGTGACAGTTGTGACAGCTACCACAGGATCAAAACAGAATT
GTACCACTGGAGAAGGTGACTATCAGTTAGTACAGCATGAAGTCTTATG
CTCCATGAAAAATACTTACGAAGTCCTTGATTTTCTTGGTCGAGGCACG
TTTGGCCAGGTAGTTAAATGCTGGAAAAGAGGGACAAATGAAATTGTAG
CAATCAAAATTTTGAAGAATCATCCTTCTTATGCCCGTCAAGGTCAAAT
AGAAGTGAGCATATTAGCAAGGCTCAGTACTGAAAATGCTGATGAATAT
AACTTTGTACGAGCTTATGAATGCTTTCAGCACCGTAACCATACTTGTT
TAGTCTTTGAGATGCTGGAACAAAACTTGTATGACTTTCTGAAACAAAA
TAAATTTAGTCCCCTGCCACTAAAAGTGATTCGGCCCATTCTTCAACAA
GTGGCCACTGCACTGAAAAAATTGAAAAGTCTTGGTTTAATTCATGCTG
ATCTCAAGCCAGAGAATATTATGTTGGTGGATCCTGTTCGGCAGCCTTA
CAGGGTTAAAGTAATAGACTTTGGGTCGGCCAGTCATGTATCAAAGACT
GTTTGTTCAACATATCTACAATCTCGGTACTACAGAGCTCCAGAGATTA
TATTGGGGTTGCCATTTTGTGAAGCCATAGACATGTGGTCATTGGGATG
TGTGATTGCAGAATTATTTCTTGGATGGCCGCTCTACCCAGGAGCCTTG
GAGTATGATCAGATTCGATACATTTCTCAGACTCAAGGTTTGCCAGGAG
AACAGTTGTTAAATGTGGGTACTAAATCCACAAGATTTTTTTGCAAAGA
AACAGATATGTCTCATTCTGGTTGGAGATTAAAGACATTGGAAGAGCAT
GAGGCAGAGACAGGAATGAAGTCTAAAGAAGCCAGAAAATACATTTTCA
ACAGTCTGGATGATGTAGCGCATGTGAACACAGTGATGGATTTGGAAGG
AAGTGATCTTTTGGCTGAGAAAGCTGATAGAAGAGAATTTGTTAGTCTG
TTGAAGAAAATGTTGCTGATTGATGCAGATTTAAGAATTACTCCAGCTG
AGACCCTGAACCATCCTTTTGTTAATATGAAACATCTTCTAGATTTCCC
TCATAGCAACCATGTAAAGTCCTGTTTTCATATTATGGATATTTGTAAG
TCCCACCTAAATTCATGTGACACAAATAATCACAACtgagaattc