Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active HIPK1 [158 – 555]

Enzyme description:- HIPK1 [158 - 555]

Clone number:- DU 5523

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 72, 954.03 daltons
Average Mass 73, 001.33 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6,54

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

HIPK1 [158 - 555]

Protein	HIPK1 [158 - 555]
Clone number	DU 5523
Species	Human
Accession number	NM_198268
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**TTTTVTTKSSS** **SSGEGDYQLVQHEILCSMTNSYEVLEFLGRGTFGQVAKCWKRSTKEIVA** **IKILKNHPSYARQGQIEVSILSRLSSENADEYNFVRSYECFQHKNHTCL** **VFEMLEQNLYDFLKQNKFSPLPLKYIRPILQQVATALMKLKSLGLIHAD** **LKPENIMLVDPVRQPYRVKVIDFGSASHVSKAVCSTYLQSRYYRAPEII** **LGLPFCEAIDMWSLGCVIAELFLGWPLYPGASEYDQIRYISQTQGLPAE** **YLLSAGTKTTRFFNRDPNLGYPLWRLKTPEEHELETGIKSKEARKYIFN** **CLDDMAQVNMSTDLEGTDMLAEKADRREYIDLLKKMLTIDADKRITPLK** **TLNHQFVTMTHLLDFPHSNHVKSCFQNMEICKRRVHMYDTVSQI**
Native sequence	Amino acids T158 – I555 of human HIPK1. [Full length protein ends at residue L1210] Residue T235 of the fusion protein is equivalent to T158 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

gaattcACAACCACCACTGTGACCACAAAGAGTAGCAGTTCCAGCGGAG
AAGGGGATTACCAGCTGGTCCAGCATGAGATCCTTTGCTCTATGACCAA
TAGCTATGAAGTCTTGGAGTTCCTAGGCCGGGGGACATTTGGACAGGTG
GCTAAGTGCTGGAAGAGGAGCACCAAGGAAATTGTGGCTATTAAAATCT
TGAAGAACCACCCCTCCTATGCCAGACAAGGACAGATTGAAGTGAGCAT
CCTTTCCCGCCTAAGCAGTGAAAATGCTGATGAGTATAATTTTGTCCGT
TCATACGAGTGCTTTCAGCATAAGAATCACACCTGCCTTGTTTTTGAAA
TGTTGGAGCAGAACTTATATGATTTTCTAAAGCAAAACAAATTTAGCCC
ACTGCCACTCAAGTACATCAGACCAATCTTGCAGCAGGTGGCCACAGCC
TTGATGAAGCTCAAGAGTCTTGGTCTGATCCACGCTGACCTTAAGCCTG
AAAACATCATGCTGGTTGATCCAGTTCGCCAGCCCTACCGAGTGAAGGT
CATTGACTTTGGTTCTGCTAGTCACGTTTCCAAAGCTGTGTGCTCAACC
TACTTACAGTCACGTTACTACAGAGCTCCTGAAATTATTCTTGGGTTAC
CATTTTGTGAAGCTATTGATATGTGGTCACTGGGCTGTGTGATAGCTGA
GCTGTTCCTGGGATGGCCTCTTTATCCTGGTGCTTCAGAATATGATCAG
ATTCGTTATATTTCACAAACACAAGGCTTGCCAGCTGAATATCTTCTCA
GTGCCGGAACAAAAACAACCAGGTTTTTCAACAGAGATCCTAATTTGGG
GTACCCACTGTGGAGGCTTAAGACACCTGAAGAACATGAACTGGAGACT
GGAATAAAATCAAAAGAAGCTCGGAAGTACATTTTTAATTGCTTAGATG
ACATGGCTCAGGTGAATATGTCTACAGACCTGGAGGGAACAGACATGTT
GGCAGAGAAGGCAGACCGAAGAGAATACATTGATCTGTTAAAGAAAATG
CTCACAATTGATGCAGATAAGAGAATTACCCCTCTAAAAACTCTTAACC
ATCAGTTTGTGACAATGACTCACCTTTTGGATTTTCCACATAGCAATCA
TGTTAAGTCTTGTTTTCAGAACATGGAGATCTGCAAGCGGAGGGTTCAC
ATGTATGATACAGTGAGTCAGATCtaggaattc