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 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

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 (LEVLFQGP) residues 221 - 228

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