Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active HIPK4 [1 - 616]

Enzyme description:- HIPK4 [1 – 616]

Clone number:- DU 5222

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

Purification method:- Ni^{2+}-NTA agarose

Calculated molecular mass:-
Monoisotopic 73, 094.50 daltons
Average Mass 73, 141.00 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.06

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-
Myelin basic protein Final concentration: 0.3 mg/ml

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Clone Data Sheet

HIPK4 [1 - 616]

Protein	HIPK4 [1 – 616]
Clone Number	DU 5222
Species	Human
Accession number	NM_144685
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMDPEF**MSTIQSETDCYDIIEVLGK GTFGEVAKGWRRSTGEMVAIKILKNDAYRNRIIKNELKLLHCMRGLDPE EAHVIRFLEFFHDALKFYLVFELLEQNLFEFQKENNFAPLPARHIRTVT LQVLTALARLKELAIIHADLKPENIMLVDQTRCPFRVKVIDFGSASIFS EVRYVKEPYIQSRFYRAPEILLGLPFCEKVDVWSLGCVMAELHLGWPLY PGNNEYDQVRYICETQGLPKPHLLHAACKAHHFFKRNPHPDAANPWQLK SSADYLAETKVRPLERRKYMLKSLDQIETVNGGSVASRLTFPDREALAE HADLKSMVELIKRMLTWESHERISPSAALRHPFVSMQQLRSAHETTHYY QLSLRSYRLSLQVEGKPPTPVVAAEDGTPYYCLAEEKEAAGMGSVAGSS PFFREEKAPGMQRAIDQLDDLSLQEAGHGLWGETCTNAVSDMMVPLKAA ITGHHVPDSGPEPILAFYSSRLAGRHKARKPPAGSKSDSNFSNLIRLSQ VSPEDDRPCRGSSWEEGEHLGASAEPLAILQRDEDGPNIDNMTMEAERP DPELFDPSSCPGEWLSEPDCTLESVRGPRAQGLPPRRSHQHGPPRGATS FLQHVTGHH**
Native sequence	Amino acids M1 – H616 (end) of human HIPK4. Residue M31 of fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Eco*R1 and *Not*1 site of pFastBAC HTa

Nucleotide sequence of insert

gaattcATGTCCACCATCCAGTCGGAGACTGACTGCTACGACATCATCG
AGGTCTTGGGCAAGGGGACCTTCGGGGAGGTAGCCAAGGGCTGGCGGCG
GAGCACGGGCGAGATGGTGGCCATCAAGATCCTCAAGAATGACGCCTAC
CGCAACCGCATCATCAAAAACGAGCTGAAGCTGCTGCACTGCATGCGAG
GCCTAGACCCTGAAGAGGCCCACGTCATCCGCTTCCTTGAGTTCTTCCA
TGACGCCCTCAAGTTCTACCTGGTCTTTGAGCTGCTGGAGCAAAACCTT
TTCGAGTTCCAGAAGGAGAACAACTTCGCGCCCCTCCCCGCCCGCCACA
TCCGTACAGTCACCCTGCAGGTGCTCACAGCCCTGGCCCGGCTCAAGGA
GCTGGCTATCATCCACGCTGATCTCAAGCCTGAGAACATCATGCTGGTG
GACCAGACCCGCTGCCCCTTCAGGGTCAAGGTGATTGACTTCGGATCCG
CCAGCATTTTCAGCGAGGTGCGCTACGTGAAGGAGCCATACATCCAGTC
GCGCTTCTACCGGGCCCCTGAGATCCTGCTGGGGCTGCCCTTCTGCGAG
AAGGTGGACGTGTGGTCCCTGGGCTGCGTCATGGCTGAGCTGCACCTGG
GCTGGCCTCTCTACCCCGGCAACAACGAGTACGACCAGGTGCGCTACAT
CTGCGAAACCCAGGGCCTGCCCAAGCCACACCTGTTGCACGCCGCCTGC
AAGGCCCACCACTTCTTCAAGCGCAACCCCCACCCTGACGCTGCCAACC
CCTGGCAGCTCAAGTCCTCGGCTGACTACCTGGCCGAGACGAAGGTGCG
CCCATTGGAGCGCCGCAAGTATATGCTCAAGTCGTTGGACCAGATTGAG
ACAGTGAATGGTGGCAGTGTGGCCAGTCGGCTAACCTTCCCTGACCGGG
AGGCGCTGGCGGAGCACGCCGACCTCAAGAGCATGGTGGAGCTGATCAA
GCGCATGCTGACCTGGGAGTCACACGAACGCATCAGCCCCAGTGCTGCC
CTGCGCCACCCCTTCGTGTCCATGCAGCAGCTGCGCAGTGCCCACGAGA
CCACCCACTACTACCAGCTCTCGCTGCGCAGCTACCGCCTCTCGCTGCA
AGTGGAGGGGAAGCCCCCCACGCCCGTCGTGGCCGCAGAAGATGGGACC
CCCTACTACTGTCTGGCTGAGGAGAAGGAGGCTGCGGGTATGGGCAGTG
TGGCCGGCAGCAGCCCCTTCTTCCGAGAGGAGAAGGCACCAGGTATGCA
AAGAGCCATCGACCAGCTGGATGACCTGAGTCTGCAGGAGGCTGGGCAT
GGGCTGTGGGGTGAGACCTGCACCAATGCGGTCTCCGACATGATGGTCC
CCCTCAAGGCAGCCATCACTGGCCACCATGTGCCCGACTCGGGCCCTGA
GCCCATCCTGGCCTTCTACAGCAGCCGCCTGGCAGGCCGCCACAAGGCC
CGCAAGCCACCTGCGGGTTCCAAGTCCGACTCCAACTTCAGCAACCTCA
TTCGGCTGAGCCAGGTCTCGCCTGAGGATGACAGGCCCTGCCGGGGCAG
CAGCTGGGAGGAAGGAGAGCATCTCGGGGCCTCTGCTGAGCCACTGGCC
ATCCTGCAGCGAGATGAGGATGGGCCCAACATTGACAACATGACCATGG
AAGCTGAGAGGCCAGACCCTGAGCTCTTCGACCCCAGCAGCTGTCCTGG
AGAATGGCTGAGTGAGCCAGACTGCACCCTGGAGAGCGTCAGGGGCCCA
CGGGCTCAGGGGCTCCCACCCCGCCGCTCCCACCAGCATGGTCCACCCC
GGGGGGCCACCAGCTTCCTCCAGCATGTCACCGGGCACCACtgagcggc
cgc