University of Dundee

Standard Operating Procedure

Preparation of active HIPK2 [165 – 564]

Enzyme description:- HIPK2 [165 - 564]

Clone number:- DU 5524

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 72, 917.84 daltons
Average Mass 72, 965.07 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6,14

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

University of Dundee

Clone Data Sheet - HIPK2 [165 - 564]

Protein	HIPK2 [165 - 564]
Clone number	DU 5524
Species	Human
Accession number	AF326592
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**TATTSTATSKN** **SGSNSEGDYQLVQHEVLCSMTNTYEVLEFLGRGTFGQVVKCWKRGTNEI** **VAIKILKNHPSYARQGQIEVSILARLSTESADDYNFVRAYECFQHKNHT** **CLVFEMLEQNLYDFLKQNKFSPLPLKYIRPVLQQVATALMKLKSLGLIH** **ADLKPENIMLVDPSRQPYRVKVIDFGSASHVSKAVCSTYLQSRYYRAPE** **IILGLPFCEAIDMWSLGCVIAELFLGWPLYPGASEYDQIRYISQTQGLP** **AEYLLSAGTKTTRFFNRDTDSPYPLWRLKTPDDHEAETGIKSKEARKYI** **FICLDDMAQVNMTTDLEGSDMLVEKADRREFIDLLKKMLTIDADKRITP** **IETLNHPFVTMTHLLDFPHSTHVKSCFQNMEICKRRVNMYDTVNQS**
Native sequence	Amino acids T165 – S564 of human HIPK2. [Full length protein ends at residue S1171] Residue T235 of the fusion protein is equivalent to T165 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

gaattcACTGCCACCACGTCTACTGCCACCTCCAAAAACAGCGGCTCCA
ACAGCGAGGGCGACTATCAGCTGGTGCAGCATGAGGTGCTGTGCTCCAT
GACCAACACCTACGAGGTCTTAGAGTTCTTGGGCCGAGGGACGTTTGGG
CAAGTGGTCAAGTGCTGGAAACGGGGCACCAATGAGATCGTGGCCATCA
AGATCCTGAAGAACCACCCATCCTATGCCCGACAAGGTCAGATTGAAGT
GAGCATCCTGGCCCGGTTGAGCACGGAGAGTGCCGATGACTATAACTTC
GTCCGGGCCTACGAATGCTTCCAGCACAAGAACCACACGTGCTTGGTCT
TCGAGATGTTGGAGCAGAACCTCTATGACTTTCTGAAGCAAAACAAGTT
TAGCCCCTTGCCCCTCAAATACATTCGCCCAGTTCTCCAGCAGGTAGCC
ACAGCCCTGATGAAACTCAAAAGCCTAGGTCTTATCCACGCTGACCTCA
AACCAGAAAACATCATGCTGGTGGATCCATCTAGACAACCATACAGAGT
CAAGGTCATCGACTTTGGTTCAGCCAGCCACGTCTCCAAGGCTGTGTGC
TCCACCTACTTGCAGTCCAGATATTACAGGGCCCCTGAGATCATCCTTG
GTTTACCATTTTGTGAGGCAATTGACATGTGGTCCCTGGGCTGTGTTAT
TGCAGAATTGTTCCTGGGTTGGCCGTTATATCCAGGAGCTTCGGAGTAT
GATCAGATTCGGTATATTTCACAAACACAGGGTTTGCCTGCTGAATATT
TATTAAGCGCCGGGACAAAGACAACTAGGTTTTTCAACCGTGACACGGA
CTCACCATATCCTTTGTGGAGACTGAAGACACCAGATGACCATGAAGCA
GAGACAGGGATTAAGTCAAAAGAAGCAAGAAAGTACATTTTCATCTGTT
TAGATGATATGGCCCAGGTGAACATGACGACAGATTTGGAAGGGAGCGA
CATGTTGGTAGAAAAGGCTGACCGGCGGGAGTTCATTGACCTGTTGAAG
AAGATGCTGACCATTGATGCTGACAAGAGAATCACTCCAATCGAAACCC
TGAACCATCCCTTTGTCACCATGACACACTTACTCGATTTTCCCCACAG
CACACACGTCAAATCATGTTTCCAGAACATGGAGATCTGCAAGCGTCGG
GTGAATATGTATGACACGGTGAACCAGAGCtaagaattc