Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CHK1 [1 – 476]

Enzyme description:- CHK1 [1 – 476]

Clone Number:- DU 578

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:- 81, 564 daltons

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,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2−mercaptoethanol, 0.1 mM EGTA, 0.02 % Brij-35,
10 mM magnesium acetate

Substrate:-
CHKtide [KKKVSRSGLYRSPSMPENLNRPR] Final concentration: 30 µM

Specific assay range:- 100 – 300 U/mg

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Clone Data Sheet

CHK1 [1 – 476]

Protein	CHK1 [1 – 476]
Clone number	DU 578
Species	Human
Accession no	AF016582
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKW RNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNML GGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFL SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLY MDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKSDLEVLFQGPLGSPEF**MAVPFVEDWDLV** **QTLGEGAYGEVQLAVNRVTEEAVAVKIVDMKRAVDCPENIK** **KEICINKMLNHENVVKFYGHRREGNIQYLFLEYCSGGELFD** **RIEPDIGMPEPDAQRFFHQLMAGVVYLHGIGITHRDIKPEN** **LLLDERDNLKISDFGLATVFRYNNRERLLNKMCGTLPYVAP** **ELLKRREFHAEPVDVWSCGIVLTAMLAGELPWDQPSDSCQE** **YSDWKEKKTYLNPWKKIDSAPLALLHKILVENPSARITIPD** **IKKDRWYNKPLKKGAKRPRVTSGGVSESPSGFSKHIQSNLD** **FSPVNSASSEENVKYSSSQPEPRTGLSLWDTSPSYIDKLVQ** **GISFSQPTCPDHMLLNSQLLGTPGSSQNPWQRLVKRMTRFF** **TKLDADKSYQCLKETCEKLGYQWKKSCMNQVTISTTDRRNN** **KLIFKVNLLEMDDKILVDFRLSKGDGLEFKRHFLKIKGKLI** **DIVSSQKVWLPAT**
Native sequence	Amino acids M1 – T476 (end) of human CHK1. ResidueM235 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located between residues 1 - 220 of the fusion protein.
Protease cleavage	PreScission site (LEVLFQGPL) residues 221-229
Cloning sites	*Eco*R1 and *Not*1 of pFastBac GST

Nucleotide sequence of insert

ATGGCAGTGCCCTTTGTGGAAGACTGGGACTTGGTGCAAACCCTGGGA
GAAGGTGCCTATGGAGAAGTTCAACTTGCTGTGAATAGAGTAACTGAA
GAAGCAGTCGCAGTGAAGATTGTAGATATGAAGCGTGCCGTAGACTGT
CCAGAAAATATTAAGAAAGAGATCTGTATCAATAAAATGCTAAATCAT
GAAAATGTAGTAAAATTCTATGGTCACAGGAGAGAAGGCAATATCCAA
TATTTATTTCTGGAGTACTGTAGTGGAGGAGAGCTTTTTGACAGAATA
GAGCCAGACATAGGCATGCCTGAACCAGATGCTCAGAGATTCTTCCAT
CAACTCATGGCAGGGGTGGTTTATCTGCATGGTATTGGAATAACTCAC
AGGGATATTAAACCAGAAAATCTTCTGTTGGATGAAAGGGATAACCTC
AAAATCTCAGACTTTGGCTTGGCAACAGTATTTCGGTATAATAATCGT
GAGCGTTTGTTGAACAAGATGTGTGGTACTTTACCATATGTTGCTCCA
GAACTTCTGAAGAGAAGAGAATTTCATGCAGAACCAGTTGATGTTTGG
TCCTGTGGAATAGTACTTACTGCAATGCTCGCTGGAGAATTGCCATGG
GACCAACCCAGTGACAGCTGTCAGGAGTATTCTGACTGGAAAGAAAAA
AAAACATACCTCAACCCTTGGAAAAAAATCGATTCTGCTCCTCTAGCT
CTGCTGCATAAAATCTTAGTTGAGAATCCATCAGCAAGAATTACCATT
CCAGACATCAAAAAAGATAGATGGTACAACAAACCCCTCAAGAAAGGG
GCAAAAAGGCCCCGAGTCACTTCAGGTGGTGTGTCAGAGTCTCCCAGT
GGATTTTCTAAGCACATTCAATCCAATTTGGACTTCTCTCCAGTAAAC
AGTGCTTCTAGTGAAGAAAATGTGAAGTACTCCAGTTCTCAGCCAGAA
CCCCGCACAGGTCTTTCCTTATGGGATACCAGCCCCTCATACATTGAT
AAATTGGTACAAGGGATCAGCTTTTCCCAGCCCACATGTCCTGATCAT
ATGCTTTTGAATAGTCAGTTACTTGGCACCCCAGGATCCTCACAGAAC
CCCTGGCAGCGGTTGGTCAAAAGAATGACACGATTCTTTACCAAATTG
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GGCTATCAATGGAAGAAAAGTTGTATGAATCAGGTTACTATATCAACA
ACTGATAGGAGAAACAATAAACTCATTTTCAAAGTGAATTTGTTAGAA
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TTGGAGTTCAAGAGACACTTCCTGAAGATTAAAGGGAAGCTGATTGAT
ATTGTGAGCAGCCAGAAGGTTTGGCTTCCTGCCACAtga