

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active CHK2 [5 – 543]

<u>Enzyme description:-</u>	CHK2 [5 – 543]
<u>Clone number:-</u>	DU 1633
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST + C-terminal His(6)
<u>Purification method:-</u>	GSH Sepharose followed by Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	<0.5 mg/L
<u>Calculated molecular mass:-</u>	89, 430 daltons
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
	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, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	-70 °C
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	
	50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc
<u>Substrate:-</u>	
	CHKtide [KKKVSRSGLYRSPSPENLNRPR] Final concentration: 250 µM
<u>Specific activity range:-</u>	2000 – 4000 U/mg

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Clone Data Sheet

CHK2 [5 – 543]

Protein CHK2 [5 – 543]

Clone number DU 1633

Species Human

Accession number NM_007194

Tags N-terminal GST tag and C-terminal His(6) tag

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLVPRGSRRASVGSHPMSRP
RRPSDVEAQQSHGSSACSQPHGSVTQSQSSSSQSQGISSSSSTSTMPNS
SQSSHSSSGTLLSLETVSTQELYSIPEDQEPEDQEPEEPTPAPWARLW
ALQDGFANLECVNDNYWFGDKSCEYCFDEPLLKRTDKYRTYSKKHFR
IFREVGPKNSYIAYIEDHSGNGTFVNTLVGKGKRRPLNNNSEIALSL
SRNKVFFVFDLTVDDQSVYPKALRDEYIMSKTLGSGACGEVKLAFERK
TCKKVAIKIISKRKFAIGSAREADPALNVETEIEILKKNHPCIIKIK
NFFDAEDYYIVLELMEGGELFDKVVGNKRLKEATCKLYFYQMLLAVQY
LHENGIHRDLKPENVLLSSQEEDCLIKITDFGHSKILGETSLMRTL
GTPTYLAPEVLVSVGTAGYNRAVDCWSLGVILFICLSGYPPFSEHRTQ
VSLKDQITSGKYNFIPEVWAEVSEKALDLVKKLLVDPKARFTTEAL
RHPWLQDEDMKRKFQDLLSEENESTALPQVLAQPSTSRKRPREGEAEG
AETTKRPAVCAAVLHHHHHH

Native sequence Amino acids S5 – L543 (end) of human CHK2.
Residue S244 of the fusion protein is equivalent to S5 of the native enzyme. The GST tag is located at residues 1 - 220 and the C-terminal His(6) tag at residues 783 - 788.

Protease cleavage Thrombin (LVPRGS) at residues 221 - 226.

Cloning sites *Nde*1 and *Eco*R1 sites of modified pGEX-2TK

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Nucleotide sequence of insert

ATGCCCATGTCGAGGCCACGAAGGCCGTCGGATGTTGAGGCTCAGCAG
TTCATGGCAGCAGTGCCTGTTACAGCCCCATGGCAGCGTTACCCAG
TCCAAGGCTCCTCCTCACAGTCCAGGGCATATCCAGCTCCTCTACC
AGCACGATGCCAACTCCAGCCAGTCTCTCACTCCAGCTCTGGGACA
CTGAGCTCCTTAGAGACAGTGTCCACTCAGGAACCTATTCTATTCT
GAGGACCAAGAACCCTGAGGACCAAGAACCCTGAGGAGCCTACCCCTGCC
CCCTGGGCTCGATTATGGGCCCTTCAGGATGGATTTGCCAATCTTGAA
TGTGTGAATGACAACACTGTTTGGGAGGGACAAAAGCTGTGAATAT
TGCTTTGATGAACCACTGCTGAAAAGAACAGATAAATACCGAACATAC
AGCAAGAAACACTTTCGGATTTTCAGGGAAAGTGGGTCTAAAACTCT
TACATTGCATACATAGAAGATCACAGTGGCAATGGAACCTTTGTAAAT
ACAGAGCTTGTAGGGAAAGGAAAACGCCGTCTTTGAATAACAATTCT
GAAATTGCACTGTCACTAAGCAGAAATAAAGTTTTTGTCTTTTTTGAT
CTGACTGTAGATGATCAGTCACTTATCCTAAGGCATTAAGAGATGAA
TACATCATGTCAAAAACCTTTGGAAGTGGTGCCTGTGGAGAGGTAAAG
CTGGCTTTCGAGAGGAAAACATGTAAGAAAGTAGCCATAAAGATCATC
AGCAAAAGGAAGTTTGTATTGGTTCAGCAAGAGAGGCAGACCCAGCT
CTCAATGTTGAAACAGAAATAGAAATTTTGAAAAGCTAAATCATCCT
TGCATCATCAAGATTAAAAACCTTTTTTGTATGCAGAAGATTATTATATT
GTTTTGGAATTGATGGAAGGGGGAGAGCTGTTTGACAAAAGTGGTGGGG
AATAAACGCCTGAAAGAAGCTACCTGCAAGCTCTATTTTTACCAGATG
CTCTTGGCTGTGCAGTACCCTCATGAAAACGGTATTATACACCGTGAC
TTAAAGCCAGAGAATGTTTTACTGTCTCATCTCAAGAAGAGGACTGTCTT
ATAAAGATTACTGATTTTGGGCACCTCCAAGATTTTGGGAGAGACCTCT
CTCATGAGAACCTTATGTGGAACCCACCTACTTGGCGCCTGAAGTT
CTTGTTTCTGTTGGGACTGCTGGGTATAACCGTGCTGTGGACTGCTGG
AGTTTAGGAGTTATTCTTTTTATCTGCCTTAGTGGGTATCCACCTTTC
TCTGAGCATAGGACTCAAGTGTCACTGAAGGATCAGATCACCAGTGGAA
AAATACAACCTTCACTCCTGAAGTCTGGGCAGAAGTCTCAGAGAAAGCT
CTGGACCTTGTCAAGAAGTTGTTGGTAGTGGATCCAAAGGCACGTTTT
ACGACAGAAGAAGCCTTAAGACACCCGTGGCTTCAGGATGAAGACATG
AAGAGAAAGTTTCAAGATCTTCTGTCTGAGGAAAATGAATCCACAGCT
CTACCCAGGTTCTAGCCAGCCTTCTACTAGTCGAAAGCGGCCCGT
GAAGGGGAAGCCGAGGGTGCCGAGACCACAAAGCGCCAGCTGTGTGT
GCTGCTGTGTTGCATCACCATCACCATCACTga