Standard Operating Procedure

Preparation of active CHK2 [5 – 543]

Enzyme description:- CHK2 [5 – 543]

Clone number:- DU 1633

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST + C-terminal His(6)

Purification method:- GSH Sepharose followed by Ni^{2+}-NTA agarose

Expression level:- <0.5 mg/L

Calculated molecular mass:- 89, 430 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, 0.2 mM PMSF, 1 mM Benzamidine.

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, 10 mM MgAc

Substrate:-
CHKtide [KKKVSRSGLYRSPSMPENLNRPR] Final concentration: 250 μM

Specific activity range:- 2000 – 4000 U/mg

University of Dundee

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

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSRRASVGSHMPMSRP
RRP**SDVEAQQSHGSSACSQPHGSVTQSQGSSSQSQGISSSSTSTMPNS**
SQSSHSSSGTLSSLETVSTQELYSIPEDQEPEDQEPEEPTPAPWARLW
ALQDGFANLECVNDNYWFGRDKSCEYCFDEPLLKRTDKYRTYSKKHFR
IFREVGPKNSYIAYIEDHSGNGTFVNTELVGKGKRRPLNNNSEIALSL
SRNKVFVFFDLTVDDQSVYPKALRDEYIMSKTLGSGACGEVKLAFERK
TCKKVAIKIISKRKFAIGSAREADPALNVETEIEILKKLNHPCIIKIK
NFFDAEDYYIVLELMEGGELFDKVVGNKRLKEATCKLYFYQMLLAVQY
LHENGIIHRDLKPENVLLSSQEEDCLIKITDFGHSKILGETSLMRTLC
GTPTYLAPEVLVSVGTAGYNRAVDCWSLGVILFICLSGYPPFSEHRTQ
VSLKDQITSGKYNFIPEVWAEVSEKALDLVKKLLVVDPKARFTTEEAL
RHPWLQDEDMKRKFQDLLSEENESTALPQVLAQPSTSRKRPREGEAEG
AETTKRPAVCAAVLHHHHHH

Native sequence Amino acids S5 – L543 (end) of human CHK2.
Residue S244 of the fusion protein is equilivalent 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

Nucleotide sequence of insert

ATGCCCATGTCGAGGCCACGAAGGCCGTCGGATGTTGAGGCTCAGCAG
TCTCATGGCAGCAGTGCCTGTTCACAGCCCCATGGCAGCGTTACCCAG
TCCCAAGGCTCCTCCTCACAGTCCCAGGGCATATCCAGCTCCTCTACC
AGCACGATGCCAAACTCCAGCCAGTCCTCTCACTCCAGCTCTGGGACA
CTGAGCTCCTTAGAGACAGTGTCCACTCAGGAACTCTATTCTATTCCT
GAGGACCAAGAACCTGAGGACCAAGAACCTGAGGAGCCTACCCCTGCC
CCCTGGGCTCGATTATGGGCCCTTCAGGATGGATTTGCCAATCTTGAA
TGTGTGAATGACAACTACTGGTTTGGGAGGGACAAAAGCTGTGAATAT
TGCTTTGATGAACCACTGCTGAAAAGAACAGATAAATACCGAACATAC
AGCAAGAAACACTTTCGGATTTTCAGGGAAGTGGGTCCTAAAAACTCT
TACATTGCATACATAGAAGATCACAGTGGCAATGGAACCTTTGTAAAT
ACAGAGCTTGTAGGGAAAGGAAAACGCCGTCCTTTGAATAACAATTCT
GAAATTGCACTGTCACTAAGCAGAAATAAAGTTTTTGTCTTTTTTGAT
CTGACTGTAGATGATCAGTCAGTTTATCCTAAGGCATTAAGAGATGAA
TACATCATGTCAAAAACTCTTGGAAGTGGTGCCTGTGGAGAGGTAAAG
CTGGCTTTCGAGAGGAAAACATGTAAGAAAGTAGCCATAAAGATCATC
AGCAAAAGGAAGTTTGCTATTGGTTCAGCAAGAGAGGCAGACCCAGCT
CTCAATGTTGAAACAGAAATAGAAATTTTGAAAAAGCTAAATCATCCT
TGCATCATCAAGATTAAAAACTTTTTTGATGCAGAAGATTATTATATT
GTTTTGGAATTGATGGAAGGGGGAGAGCTGTTTGACAAAGTGGTGGGG
AATAAACGCCTGAAAGAAGCTACCTGCAAGCTCTATTTTTACCAGATG
CTCTTGGCTGTGCAGTACCTTCATGAAAACGGTATTATACACCGTGAC
TTAAAGCCAGAGAATGTTTTACTGTCATCTCAAGAAGAGGACTGTCTT
ATAAAGATTACTGATTTTGGGCACTCCAAGATTTTGGGAGAGACCTCT
CTCATGAGAACCTTATGTGGAACCCCCACCTACTTGGCGCCTGAAGTT
CTTGTTTCTGTTGGGACTGCTGGGTATAACCGTGCTGTGGACTGCTGG
AGTTTAGGAGTTATTCTTTTTATCTGCCTTAGTGGGTATCCACCTTTC
TCTGAGCATAGGACTCAAGTGTCACTGAAGGATCAGATCACCAGTGGA
AAATACAACTTCATTCCTGAAGTCTGGGCAGAAGTCTCAGAGAAAGCT
CTGGACCTTGTCAAGAAGTTGTTGGTAGTGGATCCAAAGGCACGTTTT
ACGACAGAAGAAGCCTTAAGACACCCGTGGCTTCAGGATGAAGACATG
AAGAGAAAGTTTCAAGATCTTCTGTCTGAGGAAAATGAATCCACAGCT
CTACCCCAGGTTCTAGCCCAGCCTTCTACTAGTCGAAAGCGGCCCCGT
GAAGGGGAAGCCGAGGGTGCCGAGACCACAAAGCGCCCAGCTGTGTGT
GCTGCTGTGTTGCATCACCATCACCATCACtga