

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of CDC6 [2 - 560]

Enzyme description:- CDC6 [2 – 560]

Clone number:- DU 1457

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 89, 356.11 daltons

Average Mass 89, 413.25 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.23

Purity:- >80 %

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

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Clone Data Sheet

CDC6 [2 – 560]

<u>Protein</u>	CDC6 [2 – 560]
<u>Clone number</u>	DU 1457
<u>Species</u>	Human
<u>Accession number</u>	NM_001254
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNM LGGCPKERA EISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIE AIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG PLGSPQTRSQAQATISFP KRKLSRALNKAKNSSDAKLEPTNVQTVTCSPRVKALPLSPRKRLGDDNL CNTPHLPPCSPPKQGKKENGPPHSHTLKGRRLVFDNQLTIKSPSKRELA KVHQNKILSSVRKSQEITTNSEQRCPLKKESACVRLFKQEGTCYQQAKL VLNTAVPDRLPAREREMDVIRNFLREHICGKKAGSLYLSGAPGTGKTAC LSRILODLKKELKGFKTIMLNCMSLRTAQAVFPAIAQEQEEVSRPAG KDMMRKLEKHMTAEKGPMIVLVLEMDQLDSKGQDVLYTLFEWPWLSNS HLVLIGIANTLDLTDRI LPR LQAREKCKPQLLNFP PYTRNQIVTILQDR LNQVSRDQVLDNAAVQFCARKVSAVSGDVRKALDVCRR AIEIVESDVKS QTILKPLSECKSPSEPLIPKRVGLIHISQVISEVDGNRMTLSQEGAQDS FPLQOKILVCSLMLLIRQLKIKEVT LGKLYEAYSKVCRKQOVAVDQSE CLSLSGLLEARGILGLKR NKETRLTKVFFKIEEKEIEHALKD KALIGNI LATGLP
<u>Native sequence</u>	Amino acids P2 – P560 (end) of human CDC6. Residue P232 of the fusion protein is equivalent to P2 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccCCTCAAACCCGATCCCAGGCACAGGCTACAATCAGTTTTCCAA
AAAGGAAGCTGTCTCGGGCATTGAACAAAGCTAAAACTCCAGTGATGC
CAAAC TAGAACCAACAAATGTCCAAACCGTAACCTGTTCTCCTCGTGTA
AAAGCCCTGCCTCTCAGCCCCAGGAAACGTCTGGGCGATGACAACCTAT
GCAACACTCCCCATTTACCTCCTTGTTCTCCACCAAAGCAAGGCAAGAA
AGAGAATGGTCCCCCTCACTCACATACACTTAAGGGACGAAGATTGGTA
TTTGACAATCAGCTGACAATTAAGTCTCCTAGCAAAAGAGAACTAGCCA
AAGTTCACCAAAACAAAATACTTTCTTCAGTTAGAAAAAGTCAAGAGAT
CACAACAAATTCTGAGCAGAGATGTCCACTGAAGAAAGAATCTGCATGT
GTGAGACTATTCAAGCAAGAAGGCACCTTGCTACCAGCAAGCAAAGCTGG
TCCTGAACACAGCTGTCCCAGATCGGCTGCCTGCCAGGGAAAGGGAGAT
GGATGTCATCAGGAATTTCTTGAGGGAACACATCTGTGGGAAAAAAGCT
GGAAGCCTTTACCTTTCTGGTGCTCCTGGAAGTGGAAAACTGCCTGCT
TAAGCCGGATTCTGCAAGACCTCAAGAAGGAACTGAAAGGCTTTAAAC
TATCATGCTGAATTGCATGTCTTGAGGACTGCCCAGGCTGTATTCCCA
GCTATTGCTCAGGAGATTTGTGAGGAAGAGGTATCCAGGCCAGCTGGGA
AGGACATGATGAGGAAATTGGAAAAACATATGACTGCAGAGAAGGGCCC
CATGATTGTGTTGGTATTGGACGAGATGGATCAACTGGACAGCAAAGGC
CAGGATGTATTGTACACGCTATTTGAATGGCCATGGCTAAGCAATTCTC
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TTAATCAGGTATCTAGAGATCAGGTTCTGGACAATGCTGCAGTTCAATT
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GATTCCCAAGAGGGTTGGTCTTATTCACATATCCCAAGTCATCTCAGAA
GTTGATGGTAACAGGATGACCTTGAGCCAAGAAGGAGCACAAGATTCCCT
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GTTTGTCACTTTCAGGGCTCTTGGAAGCCAGGGGCATTTTAGGATTAAA
GAGAAACAAGGAAACCCGTTTGACAAAGGTGTTTTTCAAGATTGAAGAG
AAAGAAATAGAACATGCTCTGAAAGATAAAGCTTTAATTGGAAATATCT
TAGCTACTGGATTGCCTtaagcggccgc

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