

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of CCDC6 [1 - 474]

Enzyme description:- CCDC6 [1 – 474]

Clone number:- DU 14175

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 80,067.51 daltons

Average Mass 80,118.12 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.54

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

CCDC6 [1 - 474]

<u>Protein</u>	CCDC6 [1 - 474]
<u>Clone number</u>	DU 14175
<u>Species</u>	Human
<u>Accession number</u>	BC064391.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLOGWQATFGGGDHPKSDLEVLFGQPLGSMADSASESD TDGAGGNSSSSAAMQSSCSSTSGGGGGGGGGGGGGKSGGIVISPFRL ELTNRLASLQQENKVLKIELETYKLCKALQEEENRDLRKASVTIQARA EQEEKFISNTLFKKIQALQKEKETLAVNYEKEEEFLTNELSRKLMQLQ HEKAELEQHLEQEQEFQVNKLMKKIKKLENDTISKQLTLEQLRREKID LENTLEQEQEALVNRLWKRMDKLEAEKRILQEKLDQPVSAAPPSPRDIS MEIDSPENMMRHIRFLKNEVERLKKQLRAAQLOHSEKMAQYLEEERHM REENLRLQKLOREMERREALCRQLSESESSLEMDDERYFNEMSAQGL RPRTVSSPIPYTPSPSSSRPISPGLSYASHTVGFTPPTSLTRAGMSYY NSPGLHVQHMGTSHGITRPSRRSNSPDKFKRPTPPSPNTQTPVQPP PPPPPPMQPTVPSAATSQPTPSQHSHTSSQP
<u>Native sequence</u>	Amino acids M1 – P474 (end) of human CCDC6. Residue M232 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	Prescission site (<u>LEVLFQGP</u>) at 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

ggatccATGGCGGACAGCGCCAGCGAGAGCGACACGGACGGGGCGGGG
GGCAACAGCAGCAGCTCGGCCGCCATGCAGTCGTCCTGCTCGTCGACC
TCGGGCGGCGGCGGTGGCGGCGGGGAGGCGGCGGCGGTGGGAAGTCG
GGGGGCATTGTCATCTCGCCGTTCCGCCTGGAGGAGCTCACCAACCGC
CTGGCCTCGCTGCAGCAAGAGAACAAGGTGCTGAAGATAGAGCTGGAG
ACCTACAAACTGAAGTGCAAGGCACTGCAGGAGGAGAACCGCGACCTG
CGCAAAGCCAGCGTTACCATCCAAGCCAGGGCTGAGCAGGAAGAAGAA
TTCATTAGTAACACTTTTATTCAAGAAAATTCAGGCTTTGCAGAAGGAG
AAAGAAACCCTTGCTGTAAATTATGAGAAAGAAGAAGAATTCCCTCACT
AATGAGCTCTCCAGAAAATTGATGCAGTTGCAGCATGAGAAAGCCGAA
CTAGAACAGCATCTTGAACAAGAGCAGGAATTTTCAGGTCAACAAACTG
ATGAAGAAAATTAAAAAACTGGAGAATGACACCATTTCTAAGCAACTT
ACATTAGAACAGTTGAGACGGGAGAAGATTGACCTTGAAAATACATTG
GAACAAGAACAAGAAGCACTAGTTAATCGCCTCTGGAAAAGGATGGAT
AAGCTTGAAGCTGAAAAGCGAATCCTGCAGGAAAAATTAGACCAGCCC
GTCTCTGCTCCACCATCGCCTAGAGATATCTCCATGGAGATTGATTCT
CCAGAAAATATGATGCGTCACATCAGGTTTTTTAAAGAATGAAGTGGA
CGGCTGAAGAAGCAACTGAGAGCTGCTCAGTTACAGCATTCAGAGAAA
ATGGCACAGTATCTGGAGGAGGAACGTCACATGAGAGAAGAGAAGCTTG
AGGCTCCAGAGGAAGCTGCAGAGGGAGATGGAGAGAAGAGAAGCCCTC
TGTCGACAGCTCTCCGAGAGTGAGTCCAGCTTAGAAATGGACGACGAA
AGGTATTTTAATGAGATGTCTGCACAAGGATTAAGACCTCGCACTGTG
TCCAGCCCGATCCCTTACACACCTTCTCCGAGTTCAAGCAGGCCTATA
TCACCTGGTCTATCATATGCAAGTCACACGGTTGGTTTCACGCCACCA
ACTTCACTGACTAGAGCTGGAATGTCTTATTACAATTCCCCGGGTCTT
CACGTGCAGCACATGGGAACATCCCATGGTATCACAAGGCCTTCACCA
CGGAGAAGCAACAGTCCTGACAAATTCAAACGGCCACGCCGCCTCCA
TCTCCCAACACACAGACCCAGTCCAGCCACCTCCACCTCCACCTCCG
CCACCCATGCAGCCACGGTCCCCCTCAGCAGCCACCTCGCAGCCTACT
CCTTCGCAACATTTCGGCGCACACCTCCTCCCAGCCTtaagcggccgc