

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of CDC73 [1 - 531]

Enzyme description:- CDC73 [1 – 531]

Clone number:- DU 17784

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 87, 718.88 daltons

Average Mass 87, 774.16daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.07

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

CDC73 [1 - 531]

Protein CDC73 [1 - 531]

Clone number DU 17784

Species Human

Accession number NM_024529.4

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEHLIERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMDCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSPEFMADVLS
VLRQYNIQKKEIVVKGDEVIFGEFSWPKNVKTNYVVWGTGKEGQPREY
YTLDSILFLLNNVHLSHPVYVRRRAATENIPVRRPDRKDLLGYLNGEA
STSASIDRSAPLEIGLQSTQVKRADEVLAEAKKPRIEDECVRDLK
ERLAARLEGHKEGIVQTEQIRSLSEAMSVEKIAAIKAKIMAKKRSTIK
TDLDDITALKQRSFVDAEVDVTRDIVSRERVWRTRTTILOSTGKNFS
KNIFAILQSVKAREEGRAPEQRPAPNAAPVDPTLRTKQPIPAAYNRYD
QERFKGKEETEGFKIDTMGTYHGMLKSVTEGASARKTQTPAAQVPR
PVSQARPPPNQKKSRTPIIIIPAATTSLITMLNAKDLLQDLKFVPSD
EKKKQGCQRENETLIQRRKDQMPGGTAISVTVPYRVVDQPLKMPQD
WDRVAVFVQGPWQFKGWPWLLPDGSPVDIFAKIKAFHLKYDEVRLD
PNVQKWDVTVLELSYHKRHLDRPVFLRFWETLDRYMVKHKSHLRF

Native sequence Amino acids M1 – F531 (end) of human CDC73.
Residue M235 of fusion protein is equivalent to M1 of the native
enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage Precission site (LEVLFOGP) at residues 221 – 228

Cloning sites *Eco*R1 and *Not*I sites of pGex6P-1

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**Nucleotide
sequence of insert**

gaattcATGGCGGACGTGCTTAGCGTCCTGCGACAGTACAACATCCAG
AAGAAGGAGATTGTGGTGAAGGGAGACGAAGTGATCTTCGGGGAGTTC
TCCTGGCCCAAGAATGTGAAGACCAACTATGTTGTTTGGGGGACTGGA
AAGGAAGGCCAACCCAGAGAGTACTACACATTGGATTCCATTTTATTT
CTACTTAATAACGTGCACCTTTCTCATCTGTTTATGTCCGACGTGCA
GCTACTGAAAATATTCCTGTGGTTAGAAGACCTGATCGAAAAGATCTA
CTTGGATATCTCAATGGTGAAGCGTCAACATCGGCAAGTATAGACAGA
AGCGCTCCCTTAGAAATAGGTCTTCAGCGATCTACTCAAGTCAAACGA
GCTGCAGATGAAGTTTTAGCAGAAGCAAAGAAACCACGAATTGAGGAT
GAAGAGTGTGTGCGCCTTGATAAAGAGAGATTGGCTGCCCGTTTGGGAT
GGTCACAAAGAAGGGATTGTACAGACTGAACAGATTAGGTCTTTGTCT
GAAGCTATGTCAGTGGAAAAAATTGCTGCAATCAAAGCCAAAAATTATG
GCTAAGAAAAGATCTACTATCAAGACTGATCTAGATGATGACATAACT
GCCCTTAAACAGAGGAGTTTTGTGGATGCTGAGGTAGATGTGACCCGA
GATATTGTCAGCAGAGAGAGAGTATGGAGGACACGAACAACATCTTA
CAAAGCACAGGAAAGAATTTTTCCAAGAACATTTTTGCAATTCCTCAA
TCTGTAAAAGCCAGAGAAGAAGGGCGTGCACCTGAACAGCGACCTGCC
CCAAATGCAGCACCTGTGGATCCCACCTTTCGCGACCAAACAGCCTATC
CCAGCTGCCATATAACAGATACGATCAGGAAAGATTCAAAGGAAAAGAA
GAAACGGAAGGCTTCAAATTTGACACTATGGGAACCTACCATGGTATG
ACACTGAAATCTGTAACGGAGGGTGCATCTGCCCGGAAGACTCAGACT
CCTGCAGCCCAGCCAGTACCAAGACCAGTTTTCTCAAGCAAGACCTCCC
CCAAATCAGAAGAAAGGATCTCGAACACCCATTATCATAATTCCTGCA
GCTACCACCTCTTTAATAACCATGCTTAATGCAAAAAGACCTTCTACAG
GACCTGAAATTTGTCCCATCAGATGAAAAGAAGAAACAAGGTTGTCAA
CGAGAAAATGAAACTCTAATACAAAGAAGAAAAGACCAGATGCAACCA
GGGGGCACTGCAATTAGTGTTACAGTACCTTATAGAGTAGTAGACCAG
CCCCTTAACTTATGCCTCAAGACTGGGACCGCGTTGTAGCCGTTTTTT
GTGCAGGGTCCCTGCATGGCAGTTCAAAGGTTGGCCATGGCTTTTGCCT
GATGGATCACCAGTTGATATATTTGCTAAAATTAAGCCTTCCATCTG
AAGTATGATGAAGTTCGTCTGGATCCAAATGTTTCAGAAATGGGATGTA
ACAGTATTAGAACTCAGCTATCACAAACGTCATTTGGATAGACCAGTG
TTCTTACGGTTTTTGGGAAACATTGGACAGGTACATGGTAAAGCATAAA
TCGCACTTGAGATTctgagcggccgc