Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of GDI2 [1 - 445]

Enzyme description:- GDI2 [1 – 445]

Clone number:- DU 52615

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 77, 437.51 daltons
Average Mass 77, 487.41 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.93

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70

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Clone Data Sheet

GDI2 [1 – 445]

Protein GDI2 [1 – 445]

Clone number DU 52615

Species Human

Accession number NM_001494.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MNEEYDVIV**
LGTGLTECILSGIMSVNGKKVLHMDRNPYYGGESASITPLEDLYKRFK
IPGSPPESMGRGRDWNVDLIPKFLMANGQLVKMLLYTEVTRYLDFKVT
EGSFVYKGGKIYKVPSTEAEALASSLMGLFEKRRFRKFLVYVANFDEK
DPRTFEGIDPKKTTMRDVYKKFDLGQDVIDFTGHALALYRTDDYLDQP
CYETINRIKLYSESLARYGKSPYLYPLYGLGELPQGFARLSAIYGGTY
MLNKPIEEIIVQNGKVIGVKSEGEIARCKQLICDPSYVKDRVEKVGQV
IRVICILSHPIKNTNDANSCQIIIPQNQVNRKSDIYVCMISFAHNVAA
QGKYIAIVSTTVETKEPEKEIRPALELLEPIEQKFVSISDLLVPKDLG
TESQIFISRTYDATTHFETTCDDIKNIYKRMTGSEFDFEEMKRKKNDI
YGED

Native sequence Amino acids M1 – D445 (end) of human GDI2.
Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*1 site of pGEX6P-1

Nucleotide sequence of insert

ggatccATGAATGAGGAGTACGACGTGATCGTGCTGGGCACCGGCCTG
ACGGAATGTATCCTGTCAGGTATAATGTCAGTGAATGGCAAGAAAGTT
CTTCATATGGATCGAAACCCTTACTACGGAGGAGAGAGTGCATCTATA
ACACCATTGGAAGATTTATACAAAAGATTTAAAATACCAGGATCACCA
CCCGAGTCAATGGGGAGAGGAAGAGACTGGAATGTTGACTTGATTCCC
AAGTTCCTTATGGCTAATGGTCAGCTGGTTAAGATGCTGCTTTATACA
GAGGTAACTCGCTATCTGGATTTTAAAGTGACTGAAGGGAGCTTTGTC
TATAAGGGTGGAAAAATCTACAAGGTTCCTTCCACTGAAGCAGAAGCC
CTGGCATCTAGCCTAATGGGATTGTTTGAAAAACGTCGCTTCAGGAAA
TTCCTAGTGTATGTTGCCAACTTCGATGAAAAAGATCCAAGAACTTTT
GAAGGCATTGATCCTAAGAAGACCACAATGCGAGATGTGTATAAGAAA
TTTGATTTGGGTCAAGACGTTATAGATTTTACTGGTCATGCTCTTGCA
CTTTACAGAACTGATGATTACTTAGATCAACCGTGTTATGAAACCATT
AATAGAATTAAACTTTACAGTGAATCTTTGGCAAGATATGGCAAAAGC
CCATACCTTTATCCACTCTATGGCCTTGGAGAACTGCCCCAAGGATTT
GCAAGGCTAAGTGCTATTTATGGAGGTACCTATATGCTGAATAAACCC
ATTGAAGAAATCATTGTACAGAATGGAAAAGTAATTGGTGTAAAATCT
GAAGGAGAAATTGCTCGCTGTAAGCAGCTCATCTGTGACCCCAGCTAC
GTAAAAGATCGGGTAGAAAAAGTGGGCCAGGTGATCAGAGTTATTTGC
ATCCTCAGCCACCCCATCAAGAACACCAATGATGCCAACTCCTGCCAG
ATCATTATTCCACAGAACCAAGTCAATCGAAAGTCAGATATCTACGTC
TGCATGATCTCCTTTGCGCACAATGTAGCAGCACAAGGGAAGTACATT
GCTATAGTTAGTACAACTGTGGAAACCAAGGAGCCTGAGAAGGAAATC
AGACCAGCTTTGGAGCTCTTGGAACCAATTGAACAGAAATTTGTTAGC
ATCAGTGACCTCCTGGTACCAAAAGACTTGGGAACAGAAAGCCAGATC
TTTATTTCCCGCACATATGATGCCACCACTCATTTTGAGACAACGTGT
GATGACATTAAAAACATCTATAAGAGGATGACAGGATCAGAGTTTGAC
TTTGAGGAAATGAAGCGCAAGAAGAATGACATCTATGGGGAAGACtaa
gcggccgc