Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of GDI1 [1 - 447]

Enzyme description:- GDI1 [1 – 447]

Clone number:- DU 52614

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 77, 356.78 daltons
Average Mass 77, 406.89 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.21

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

Division of Signal Tranduction Therapy

Clone Data Sheet

GDI1 [1 – 447]

Protein GDI1 [1 – 447]

Clone number DU 52614

Species Human

Accession number NM_001493.2

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MDEEYDVIV**
LGTGLTECILSGIMSVNGKKVLHMDRNPYYGGESSSITPLEELYKRFQ
LLEGPPESMGRGRDWNVDLIPKFLMANGQLVKMLLYTEVTRYLDFKVV
EGSFVYKGGKIYKVPSTETEALASNLMGMFEKRRFRKFLVFVANFDEN
DPKTFEGVDPQTTSMRDVYRKFDLGQDVIDFTGHALALYRTDDYLDQP
CLETVNRIKLYSESLARYGKSPYLYPLYGLGELPQGFARLSAIYGGTY
MLNKPVDDIIMENGKVVGVKSEGEVARCKQLICDPSYIPDRVRKAGQV
IRIICILSHPIKNTNDANSCQIIIPQNQVNRKSDIYVCMISYAHNVAA
QGKYIAIASTTVETTDPEKEVEPALELLEPIDQKFVAISDLYEPIDDG
CESQVFCSCSYDATTHFETTCNDIKDIYKRMAGTAFDFENMKRKQNDV
FGEAEQ

Native sequence Amino acids M1 – Q447 (end) of human GDI1.
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

ggatccATGGACGAGGAATACGATGTGATCGTGCTGGGGACCGGTCTC
ACCGAATGCATCCTGTCGGGCATCATGTCTGTGAACGGGAAGAAGGTG
CTGCACATGGACCGGAACCCCTACTACGGGGGCGAGAGCTCCTCCATC
ACACCCCTGGAGGAGCTGTATAAGCGTTTTCAGTTGCTGGAGGGGCCC
CCTGAGTCGATGGGCCGAGGCCGAGACTGGAATGTTGACCTGATTCCC
AAATTCCTCATGGCTAACGGGCAGCTGGTAAAGATGCTACTGTATACA
GAGGTGACTCGCTACCTGGACTTCAAGGTGGTGGAGGGCAGCTTTGTC
TACAAGGGGGGCAAGATCTACAAAGTGCCGTCCACTGAGACTGAGGCC
TTGGCTTCCAATCTGATGGGCATGTTTGAGAAACGGCGCTTCCGCAAG
TTCCTGGTGTTTGTGGCAAACTTCGATGAGAATGACCCCAAGACCTTT
GAGGGCGTTGACCCCCAGACTACCAGCATGCGTGACGTCTACCGGAAG
TTTGATCTGGGCCAGGATGTCATCGATTTCACTGGCCATGCCCTGGCG
CTCTACCGCACTGATGACTACCTGGACCAGCCCTGCCTTGAGACCGTC
AACCGCATCAAGTTGTACAGTGAGTCCCTGGCCCGGTATGGCAAGAGC
CCATATTTATACCCGCTCTACGGCTTGGGCGAGCTGCCCCAGGGTTTT
GCAAGATTGAGTGCCATCTATGGGGGGACATATATGCTGAACAAACCT
GTGGATGACATCATCATGGAGAACGGCAAGGTGGTGGGCGTGAAGTCT
GAGGGAGAGGTGGCCCGCTGCAAGCAGCTGATCTGTGACCCCAGCTAC
ATCCCGGACCGTGTGCGGAAGGCTGGCCAGGTTATCCGCATCATCTGT
ATCCTTAGCCACCCCATCAAGAACACCAACGACGCCAACTCCTGCCAA
ATAATCATCCCCCAGAACCAGGTCAACAGGAAGTCAGACATCTACGTG
TGCATGATCTCCTATGCACACAACGTGGCGGCCCAGGGCAAGTACATA
GCTATTGCCAGCACTACTGTGGAGACCACGGACCCTGAAAAGGAGGTG
GAGCCGGCTCTGGAGCTGTTGGAGCCCATTGACCAGAAGTTTGTGGCT
ATCAGTGACTTGTATGAGCCCATTGATGATGGTTGTGAGAGCCAGGTG
TTCTGTTCCTGCTCCTACGATGCCACCACACACTTTGAGACAACCTGC
AACGACATCAAAGACATCTACAAACGCATGGCTGGCACGGCCTTTGAC
TTTGAGAACATGAAGCGCAAACAGAACGACGTCTTTGGAGAAGCTGAG
CAGtgagcggccgc