

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of GRP1 PH domain [241 – 399]

Protein description:- GRP1 PH domain [241 – 399]

Clone number:- DU 5047

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 1 mg/L

Calculated molecular mass:- 45, 541 daltons

Purity:- 90 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 10 % glycerol, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- –20 °C

Assay:- Sensor for PIP3

Division of Signal Transduction Therapy

CLONE DATA SHEET

GRP1 PH domain [241 - 399]

<u>Protein</u>	GRP1 PH domain [241 - 399]
<u>Clone number</u>	DU 5047
<u>Species</u>	Mouse [identical to human at amino acid level]
<u>Accession no</u>	BC035296
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLRYERDEGDKW RNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNML GGCPKERAELISMLEGAVLDIRYGVSRIAYSKDFETLKVDFL SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLY MDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKSDLVPRGSPNSPFESIKNEPFKIPEDDG NDLTHTFNPDREGWLLKLGGRVKTWKRRWFILTDNCLYYF EYTTDKPRGIIIPLENLSIREVEDPRKPNCFELYNPSHKGQ VIKACKTEADGRVVEGNHVYRISAPSPEEKEEWMKSIKAS ISRDPFYDMLATRRRIANKK</p>
<u>Native sequence</u>	<p>Amino acids E241 – K399 (end) of mouse GRP1. Residue E232 of the fusion protein is equivalent to E241 of the native protein. The GST tag is located at residues 1 - 220</p>
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) residues 221 - 226
<u>Cloning sites</u>	<i>Eco</i> R1 of pGEX4T-3

Division of Signal Transduction Therapy

Complete Nucleotide sequence

atgtcccctatactaggttattggaaaattaag
ggccttgtgcaaccactcgacttcttttggaa
tatcttgaagaaaaatatgaagagcatttgtat
gagcgcgatgaaggtgataaatggcgaaacaaa
aagtttgaattgggtttggagtttcccaatctt
ccttattatattgatgggtgatgttaaattaaca
cagtctatggccatcatacgttatatagctgac
aagcacaacatggtgggtgggtgtccaaaagag
cgtgcagagatttcaatgcttgaaggagcgggt
ttggatattagatacgggtgtttcgagaattgca
tatagtaaagactttgaaactctcaaagttgat
tttcttagcaagctacctgaaatgctgaaaatg
ttcgaagatcgtttatgtcataaaacatattta
aatgggtgatcatgtaaccatcctgacttcatg
ttgatgacgctcttgatggtgttttatacatg
gaccaatgtgcctggatgcgttcccaaaatta
gtttgttttaaaaaacgtattgaagctatccca
caaattgataagtacttgaaatccagcaagtat
atagcatggcctttgcagggctggcaagccacg
tttgggtgggtggcgaccatcctccaaaatcggat
ctgGTTCCGCGTGGATCCCCGAATTCgcCCTTT
GAAAGTATCAAGAATGAGCCGTTTAAGATCCCA
GAAGACGACGGAAATGACCTGACACACACG TTC
TTCAACCCAGACCGAGAAGGCTGGCTGCTGAAG
CTGGGGGGTTCGTGTGAAGACCTGGAAACGGCGC
TGGTTCATCCTCACAGATAACTGCCTCTACTAC
TTTGAGTACACCACGGACAAGGAGCCCAGGGGC
ATCATCCCCCTGGAGAACCTCAGCATCAGGGAG
GTGGAGGACCCCCGGAAGCCGAAGTCTTTGAG
CTGTATAACCCCAGTCACAAAGGTCAAGTCATC
AAGGCCTGCAAGACGGAGCCGATGGCCGTGTG
GTGGAGGGCAACCACGTTGTGTACCGGATCTCT
GCCCCAGCCCCGGAGGAAAAGGAGGAGTGGATG
AAGTCCATCAAAGCAAGCATCAGTAGGGACCCG
TTCTATGACATGTTGGCCACGAGGAAAAGGAGG
ATTGCCAATAAGAAAtag