

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of GRB2 [1 - 217]

Enzyme description:- GRB2 [1 – 217]

Clone number:- DU 31180

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 51, 997.07 daltons

Average Mass 52, 030.52 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.81

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

Division of Signal Transduction Therapy

Clone Data Sheet

GRB2 [1 - 217]

<u>Protein</u>	GRB2 [1 - 217]
<u>Clone number</u>	DU 31180
<u>Species</u>	Human
<u>Accession number</u>	NM_002086.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWAQATFGGGDHPKSDLEVLFGQPLGSMEAIKYDF KATADDELSFKRGDILKVLNEECDQNWYKAELNGKDGFIPKNYIEMKP HPWFFGKIPRAKAEEMLSKQRHDGAFLIRESESAPGDFSLSVKFGNDV QHFKVLRDGAGKYFLWVVKFNSLNELVVDYHRSTSVSRNQQIFLRDIEQ VPQQPTYVQALFDFDPQEDGELGFRRGDFIHVMDNSDPNWWKGACHGQ TGMFPRNYVTPVNRNV
<u>Native sequence</u>	Amino acids M1 – V217 (end) of human GRB2. 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>LEVLFGQP</u>) at residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGex6P-1

Division of Signal Transduction Therapy

Nucleotide sequence of insert

ggatccATGGAAGCCATCGCCAAATATGACTTCAAAGCTACTGCAGAC
GACGAGCTGAGCTTCAAAAGGGGGACATCCTCAAGGTTTTGAACGAA
GAATGTGATCAGAAGTGGTACAAGGCAGAGCTTAATGGAAAAGACGGC
TTCATTCCCAAGAACTACATAGAAATGAAACCACATCCGTGGTTTTTT
GGCAAAATCCCCAGAGCCAAGGCAGAAGAAATGCTTAGCAAACAGCGG
CACGATGGGGCCTTTCTTATCCGAGAGAGTGAGAGCGCTCCTGGGGAC
TTCTCCCTCTCTGTCAAGTTTTGGAAACGATGTGCAGCACTTCAAGGTG
CTCCGAGATGGAGCCGGGAAGTACTTCCTCTGGGTGGTGAAGTTCAAT
TCTTTGAATGAGCTGGTGGATTATCACAGATCTACATCTGTCTCCAGA
AACCAGCAGATATTCCTGCGGGACATAGAACAGGTGCCACAGCAGCCG
ACATACGTCCAGGCCCTCTTTGACTTTGATCCCAGGAGGATGGAGAG
CTGGGCTTCCGCCGGGGAGATTTTATCCATGTCATGGATAACTCAGAC
CCCAACTGGTGGAAAGGAGCTTGCCACGGGCAGACCGGCATGTTTCCC
CGCAATTATGTCACCCCCGTGAACCGGAACGTCTaagcggccgc