

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of NRBP1 [1 - 535]

Enzyme description:- NRBP1 [1 – 535]

Clone number:- DU 68346

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 86, 613.77 daltons

Average Mass 86, 668.86 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.17

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Division of Signal Transduction Therapy

Clone Data Sheet

NRBP1 [1 – 535]

Protein NRBP1 [1 – 535]

Clone number DU 68346

Species Human

Accession number NM_013392.4

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA
VLDIRYGVSRAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMSEGESQTVLSSGS
DPKVESSSSAPGLTSVSPVVTSTTSAASPEEEEESEDESEILEESPCGR
WQKRREEVNQRNVPGIDSAYLAMDTEEGVEVWNEVQFSERKNYKLQEE
KVRAVFDNLIQLEHLNIVKFHKYWADIKENKARVIFITEYMSSGSLKQF
LKKTCKNHKTMNEKAWKRWCTQILSALSYLHSCDPPIIHGNTCDTIFI
QHGLIKIGSVAPDTINNHVKTCREEQKNLHFFAPEYGEVTNVTAVDI
YSFGMCALEMAVLEIQNGESSYVPQEAISSAIQLLEDPLQREFIQKCL
QSEPARRPTARELLFHPALFEVPSLKLAAHCIVGHQHMIPENALEEIT
KNMDTSAVLAEIPAGPGREPVTLYSQSPALEDKFLDVRNGIYPLTA
FGLPRPQQPQQEEVTSPPVPPSVKTPPEPAEVETRKVVLMLQCNIESVE
EGVKHHLTLLKLEDKLNRLSCDLMPNENIPELAAELVQLGFISEADQ
SRLTSLLEETLNKFNFARNSTLNSAAVTVSS

Native sequence Amino acids M1 – S535 (end) of human NRBP1.
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 sites of pGEX6P-1

Division of Signal Transduction Therapy

Complete Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTT
TGGAAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAAT
GGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGG
TGATGTTAAATTAACACAGTCTATGGCCATCATACTTATATAGCTGACAAGCACAACATG
TTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGAT
ATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTT
TCTTAGCAAGTACCTGAAATGCTGAAAATGTTTCAAGATCGTTTATGTCATAAAACATATT
TAAATGGTGATCATGTAACCCATCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTA
TACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTG
AAGCTATCCCACAAATTGATAAGTACTTCAAATCCAGCAAGTATATAGCATGGCCTTTGCA
GGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCTCCAAAATCGGATCTGGAAGTTCT
GTTCCAGGGGGCCCCTGGGATCCATGTCGGAGGGGGAGTCCAGACAGTACTTAGCAGTG
GCTCAGACCCAAAGGTAGAATCCTCATCTTCAGCTCCTGGCCTGACATCAGTGTACCTCC
TGTGACCTCCACAACCTCAGCTGCTTCCCCAGAGGAAGAAGAAGAAAGTGAAGATGAGTC
TGAGATTTTGAAGAGTGCCTGTGGGCGCTGGCAGAAGAGGGCGAGAAGAGGTGAATC
AACGGAATGTACCAGGTATTGACAGTGCATACCTGGCCATGGATACAGAGGAAGGTGTAG
AGGTTGTGTGGAATGAGGTACAGTTCTCTGAACGCAAGAACTACAAGCTGCAGGAGGAAA
AGGTTCTGTGTGTTTGAATACTGATTCAATTGGAGCATCTTAACATTGTTAAGTTTAC
AAATATTGGGCTGACATTAAGAGAAACAAGGCCAGGGTCATTTTTATCAGAAATACATGT
CATCTGGGAGTCTGAAGCAATTTCTGAAGAAGACCAAAAAGAACCACAAGACGATGAATG
AAAAGGCATGGAAGCGTTGGTGCACACAAATCCTCTCTGCCCTAAGCTACCTGCACTCCT
GTGACCCCCCATCATCCATGGGAACCTGACCTGTGACACCATCTTATCCAGCACAACG
GACTCATCAAGATTGGCTCTGTGGCTCCTGACACTATCAACAATCATGTGAAGACTTGTGC
AGAAGAGCAGAAGAATCTACTTCTTTGCACCAGAGTATGGAGAAGTCACTAATGTGAC
AACAGCAGTGGACATCTACTCCTTTGGCATGTGTGCACTGGAGATGGCAGTGTGGAGAT
TCAGGGCAATGGAGAGTCTCATATGTGCCACAGGAAGCCATCAGCAGTGCCATCCAGCT
TCTAGAAGACCCATTACAGAGGGAGTTCATTCAAAAGTGCCTGCAGTCTGAGCCTGCTCG
CAGACCAACAGCCAGAGAACTTCTGTTCCACCCAGCATTGTTTGAAGTGCCTCGCTCAA
CTCCTTGCGGCCCCTGCATTGTGGGACACCAACACATGATCCCAGAGAACGCTCTAGAG
GAGATCACCAAAAACATGGATACTAGTGCCGTAAGTGGCTGAAATCCCTGCAGGACCAGGA
AGAGAACCAGTTCAGACTTTGTAATCTCAGTACCAGCTCTGGAATTAGATAAAATTCCTTG
AAGATGTCAGGAATGGGATCTATCCTCTGACAGCCTTTGGGCTGCCTCGGCCCCAGCAGC
CACAGCAGGAGGAGGTGACATCACCTGTCGTGCCCCCTCTGTCAAGACTCCGACACCTG
AACCAGCTGAGGTGGAGACTCGAAGGTGGTGTGCTGATGCAGTGAACATTGAGTCGGTG
GAGGAGGGAGTCAAACACCACCTGACACTTCTGCTGAAGTTGGAGGACAACTGAACCG
GCACCTGAGCTGTGACCTGATGCCAAATGAGAATATCCCCGAGTTGGCGGCTGAGCTGGT
GCAGCTGGGCTTCATTAGTGAGGCTGACCAGAGCCGTTGACTTCTGCTAGAAGAGAC
CTTGAACAAGTTCAATTTTCCAGGAACAGTACCCTCAACTCAGCCGCTGTACCCGTCTCC
TCTtaggcggccgc