Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of NRBP1 T232A [1 - 535]

Enzyme description:- NRBP1 T232A [1 – 535]

Clone number:- DU 68840

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 86, 583.75 daltons
Average Mass 86, 638.84 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

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Clone Data Sheet

NRBP1 T232A [1 – 535]

Protein NRBP1 T232A [1 – 535]

Clone number DU 68840

Species Human

Accession number NM_013392.4

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSEGESQTVLSSGS**
DPKVESSSSAPGLTSVSPPVTSTTSAASPEEEEESEDESEILEESPCGR
WQKRREEVNQRNVPGIDSAYLAMDTEEGVEVVWNEVQFSERKNYKLQEE
KVRAVFDNLIQLEHLNIVKFHKYWADIKENKARVIFITEYMSSGSLKQF
LKKTKKNHKTMNEKAWKRWCTQILSALSYLHSCDPPIIHGNLTCDTIFI
QHNGLIKIGSVAPDTINNHVKACREEQKNLHFFAPEYGEVTNVTTAVDI
YSFGMCALEMAVLEIQGNGESSYVPQEAISSAIQLLEDPLQREFIQKCL
QSEPARRPTARELLFHPALFEVPSLKLLAAHCIVGHQHMIPENALEEIT
KNMDTSAVLAEIPAGPGREPVQTLYSQSPALELDKFLEDVRNGIYPLTA
FGLPRPQQPQQEEVTSPVVPPSVKTPTPEPAEVETRKVVLMQCNIESVE
EGVKHHLTLLLKLEDKLNRHLSCDLMPNENIPELAAELVQLGFISEADQ
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.

The enzyme has an T232A mutation. Residue T232 is equivalent to A463 of the fusion protein.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

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

Complete Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCT
GGGATCCATGTCGGAGGGGGAGTCCCAGACAGTACTTAGCAGTGGCTCA
GACCCAAAGGTAGAATCCTCATCTTCAGCTCCTGGCCTGACATCAGTGT
CACCTCCTGTGACCTCCACAACCTCAGCTGCTTCCCCAGAGGAAGAAGA
AGAAAGTGAAGATGAGTCTGAGATTTTGGAAGAGTCGCCCTGTGGGCGC
TGGCAGAAGAGGCGAGAAGAGGTGAATCAACGGAATGTACCAGGTATTG
ACAGTGCATACCTGGCCATGGATACAGAGGAAGGTGTAGAGGTTGTGTG
GAATGAGGTACAGTTCTCTGAACGCAAGAACTACAAGCTGCAGGAGGAA
AAGGTTCGTGCTGTGTTTGATAATCTGATTCAATTGGAGCATCTTAACA
TTGTTAAGTTTCACAAATATTGGGCTGACATTAAAGAGAACAAGGCCAG
GGTCATTTTTATCACAGAATACATGTCATCTGGGAGTCTGAAGCAATTT
CTGAAGAAGACCAAAAAGAACCACAAGACGATGAATGAAAAGGCATGGA
AGCGTTGGTGCACACAAATCCTCTCTGCCCTAAGCTACCTGCACTCCTG
TGACCCCCCCATCATCCATGGGAACCTGACCTGTGACACCATCTTCATC
CAGCACAACGGACTCATCAAGATTGGCTCTGTGGCTCCTGACACTATCA
ACAATCATGTGAAGgctTGTCGAGAAGAGCAGAAGAATCTACACTTCTT
TGCACCAGAGTATGGAGAAGTCACTAATGTGACAACAGCAGTGGACATC
TACTCCTTTGGCATGTGTGCACTGGAGATGGCAGTGCTGGAGATTCAGG
GCAATGGAGAGTCCTCATATGTGCCACAGGAAGCCATCAGCAGTGCCAT
CCAGCTTCTAGAAGACCCATTACAGAGGGAGTTCATTCAAAAGTGCCTG
CAGTCTGAGCCTGCTCGCAGACCAACAGCCAGAGAACTTCTGTTCCACC
CAGCATTGTTTGAAGTGCCCTCGCTCAAACTCCTTGCGGCCCACTGCAT
TGTGGGACACCAACACATGATCCCAGAGAACGCTCTAGAGGAGATCACC
AAAAACATGGATACTAGTGCCGTACTGGCTGAAATCCCTGCAGGACCAG
GAAGAGAACCAGTTCAGACTTTGTACTCTCAGTCACCAGCTCTGGAATT
AGATAAATTCCTTGAAGATGTCAGGAATGGGATCTATCCTCTGACAGCC
TTTGGGCTGCCTCGGCCCCAGCAGCCACAGCAGGAGGAGGTGACATCAC
CTGTCGTGCCCCCCTCTGTCAAGACTCCGACACCTGAACCAGCTGAGGT
GGAGACTCGCAAGGTGGTGCTGATGCAGTGCAACATTGAGTCGGTGGAG
GAGGGAGTCAAACACCACCTGACACTTCTGCTGAAGTTGGAGGACAAAC
TGAACCGGCACCTGAGCTGTGACCTGATGCCAAATGAGAATATCCCCGA
GTTGGCGGCTGAGCTGGTGCAGCTGGGCTTCATTAGTGAGGCTGACCAG
AGCCGGTTGACTTCTCTGCTAGAAGAGACCTTGAACAAGTTCAATTTTG
CCAGGAACAGTACCCTCAACTCAGCCGCTGTCACCGTCTCCTCTtaggc
ggccgc