MRC PPU REAGENTS

Standard Operating Procedure

Preparation of LRRK2 [1 - 1000] L728I

Enzyme description:- LRRK2 [1 – 1000] L728I

Clone number:- DU 27831

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 138, 946.80 daltons
Average Mass 139, 036.69 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.52

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 deg C

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Clone Data Sheet

LRRK2 [1 – 1000] L728I

Protein LRRK2 [1 – 1000] L728I

Clone number DU 27831

Species Human

Accession number NM_198578.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MASGSCQGC**
EEDEETLKKLIVRLNNVQEGKQIETLVQILEDLLVFTYSEHASKLFQG
KNIHVPLLIVLDSYMRVASVQQVGWSLLCKLIEVCPGTMQSLMGPQDV
GNDWEVLGVHQLILKMLTVHNASVNLSVIGLKTLDLLLTSGKITLLIL
DEESDIFMLIFDAMHSFPANDEVQKLGCKALHVLFERVSEEQLTEFVE
NKDYMILLSALTNFKDEEEIVLHVLHCLHSLAIPCNNVEVLMSGNVRC
YNIVVEAMKAFPMSERIQEVSCCLLHRLTLGNFFNILVLNEVHEFVVK
AVQQYPENAALQISALSCLALLTETIFLNQDLEEKNENQENDDEGEED
KLFWLEACYKALTWHRKNKHVQEAACWALNNLLMYQNSLHEKIGDEDG
HFPAHREVMLSMLMHSSSKEVFQASANALSTLLEQNVNFRKILLSKGI
HLNVLELMQKHIHSPEVAESGCKMLNHLFEGSNTSLDIMAAVVPKILT
VMKRHETSLPVQLEALRAILHFIVPGMPEESREDTEFHHKLNMVKKQC
FKNDIHKLVLAALNRFIGNPGIQKCGLKVISSIVHFPDALEMLSLEGA
MDSVLHTLQMYPDDQEIQCLGLSLIGYLITKKNVFIGTGHLLAKILVS
SLYRFKDVAEIQTKGFQTILAILKLSASFSKLLVHHSFDLVIFHQMSS
NIMEQKDQQFLNLCCKCFAKVAMDDYLKNVMLERACDQNNSIMVECIL
LLGADANQAKEGSSLICQVCEKESSPKLVELLLNSGSREQDVRKALTI
SIGKGDSQIISLLLRRLALDVANNSICLGGFCIGKVEPSWLGPLFPDK
TSNLRKQTNIASTLARMVIRYQMKSAVEEGTASGSDGNFSEDVLSKFD
EWTFIPDSSMDSVFAQSDDLDSEGSEGSFLVKKKSNSISVGEFYRDAV
LQRCSPNLQRHSNSLGPIFDHEDLLKRKRKILSSDDSLRSSKLQSHMR
HSDSISSLASEREYITSLDLSANELRDIDAL

Native sequence

Amino acids M1 – L1000 of human LRRK2 (end residue E2527). 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 a L728I mutation. Residue L728 is equivalent to I959 of the fusion protein.

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Protease cleavage PreScission (LEVLFQGP) residues 221 – 228

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

Nucleotide Sequence Of Insert

ggatccATGGCTAGTGGCAGCTGTCAGGGGTGCGAAGAGGACGAGGAAACTCTGAAGAAGTTGATAGTCAGGCTGAAC
AATGTCCAGGAAGGAAAACAGATAGAAACGCTGGTCCAAATCCTGGAGGATCTGCTGGTGTTCACGTACTCCGAGCAC
GCCTCCAAGTTATTTCAAGGCAAAAATATCCATGTGCCTCTGTTGATCGTCTTGGACTCCTATATGAGAGTCGCGAGT
GTGCAGCAGGTGGGTTGGTCACTTCTGTGCAAATTAATAGAAGTCTGTCCAGGTACAATGCAAAGCTTAATGGGACCC
CAGGATGTTGGAAATGATTGGGAAGTCCTTGGTGTTCACCAATTGATTCTTAAAATGCTAACAGTTCATAATGCCAGT
GTAAACTTGTCAGTGATTGGACTGAAGACCTTAGATCTCCTCCTAACTTCAGGTAAAATCACCTTGCTGATATTGGAT
GAAGAAAGTGATATTTTCATGTTAATTTTTGATGCCATGCACTCATTTCCAGCCAATGATGAAGTCCAGAAACTTGGA
TGCAAAGCTTTACATGTGCTGTTTGAGAGAGTCTCAGAGGAGCAACTGACTGAATTTGTTGAGAACAAAGATTATATG
ATATTGTTAAGTGCGTTAACAAATTTTAAAGATGAAGAGGAAATTGTGCTTCATGTGCTGCATTGTTTACATTCCCTA
GCGATTCCTTGCAATAATGTGGAAGTCCTCATGAGTGGCAATGTCAGGTGTTATAATATTGTGGTGGAAGCTATGAAA
GCATTCCCTATGAGTGAAAGAATTCAAGAAGTGAGTTGCTGTTTGCTCCATAGGCTTACATTAGGTAATTTTTTCAAT
ATCCTGGTATTAAACGAAGTCCATGAGTTTGTGGTGAAAGCTGTGCAGCAGTACCCAGAGAATGCAGCATTGCAGATC
TCAGCGCTCAGCTGTTTGGCCCTCCTCACTGAGACTATTTTCTTAAATCAAGATTTAGAGGAAAAGAATGAGAATCAA
GAGAATGATGATGAGGGGGAAGAAGATAAATTGTTTTGGCTGGAAGCCTGTTACAAAGCATTAACGTGGCATAGAAAG
AACAAGCACGTGCAGGAGGCCGCATGCTGGGCACTAAATAATCTCCTTATGTACCAAAACAGTTTACATGAGAAGATT
GGAGATGAAGATGGCCATTTCCCAGCTCATAGGGAAGTGATGCTCTCCATGCTGATGCATTCTTCATCAAAGGAAGTT
TTCCAGGCATCTGCGAATGCATTGTCAACTCTCTTAGAACAAAATGTTAATTTCAGAAAAATACTGTTATCAAAAGGA
ATACACCTGAATGTTTTGGAGTTAATGCAGAAGCATATACATTCTCCTGAAGTGGCTGAAAGTGGCTGTAAAATGCTA
AATCATCTTTTTGAAGGAAGCAACACTTCCCTGGATATAATGGCAGCAGTGGTCCCCAAAATACTAACAGTTATGAAA
CGTCATGAGACATCATTACCAGTGCAGCTGGAGGCGCTTCGAGCTATTTTACATTTTATAGTGCCTGGCATGCCAGAA
GAATCCAGGGAGGATACAGAATTTCATCATAAGCTAAATATGGTTAAAAAACAGTGTTTCAAGAATGATATTCACAAA
CTGGTCCTAGCAGCTTTGAACAGGTTCATTGGAAATCCTGGGATTCAGAAATGTGGATTAAAAGTAATTTCTTCTATT
GTACATTTTCCTGATGCATTAGAGATGTTATCCCTGGAAGGTGCTATGGATTCAGTGCTTCACACACTGCAGATGTAT
CCAGATGACCAAGAAATTCAGTGTCTGGGTTTAAGTCTTATAGGATACTTGATTACAAAGAAGAATGTGTTCATAGGA
ACTGGACATCTGCTGGCAAAAATTCTGGTTTCCAGCTTATACCGATTTAAGGATGTTGCTGAAATACAGACTAAAGGA
TTTCAGACAATCTTAGCAATCCTCAAATTGTCAGCATCTTTTTCTAAGCTGCTGGTGCATCATTCATTTGACTTAGTA
ATATTCCATCAAATGTCTTCCAATATCATGGAACAAAAGGATCAACAGTTTCTAAACCTCTGTTGCAAGTGTTTTGCA
AAAGTAGCTATGGATGATTACTTAAAAAATGTGATGCTAGAGAGAGCGTGTGATCAGAATAACAGCATCATGGTTGAA
TGCATTCTTCTATTGGGAGCAGATGCCAATCAAGCAAAGGAGGGATCTTCTTTAATTTGTCAGGTATGTGAGAAAGAG
AGCAGTCCCAAATTGGTGGAACTCTTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGC
ATTGGGAAAGGTGACAGCCAGATCATCAGCTTGCTCTTAAGGAGGCTGGCCCTGGATGTGGCCAACAATAGCATTTGC
CTTGGAGGATTTTGTATAGGAAAAGTTGAACCTTCTTGGCTTGGTCCTTTATTTCCAGATAAGACTTCTAATTTAAGG
AAACAAACAAATATAGCATCTACACTAGCAAGAATGGTGATCAGATATCAGATGAAAAGTGCTGTGGAAGAAGGAACA
GCCTCAGGCAGCGATGGAAATTTTTCTGAAGATGTGCTGTCTAAATTTGATGAATGGACCTTTATTCCTGACTCTTCT
ATGGACAGTGTGTTTGCTCAAAGTGATGACCTGGATAGTGAAGGAAGTGAAGGCTCATTTCTTGTGAAAAAGAAATCT
AATTCAATTAGTGTAGGAGAATTTTACCGAGATGCCGTATTACAGCGTTGCTCACCAAATTTGCAAAGACATTCCAAT
TCCTTGGGGCCCATTTTTGATCATGAAGATTTACTGAAGCGAAAAAGAAAAATACTATCTTCAGATGATTCACTCAGG
TCATCAAAACTTCAATCCCATATGAGGCATTCAGACAGCATTTCTTCTCTGGCTTCTGAGAGAGAATATATTACATCA
CTAGACCTTTCAGCAAATGAACTAAGAGATATTGATGCCCTATAGCGGCCGC