

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of LRRK2 [1 - 1000]

Enzyme description:- LRRK2 [1 – 1000]

Clone number:- DU 10534

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 139, 004.81 daltons

Average Mass 139, 094.72 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.49

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]

Protein LRRK2 [1 – 1000]

Clone number DU 10534

Species Human

Accession number NM_198578.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE
GAVLDIRYGVSRIAYSADFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMASDSCQGC
EDEEETLKKLIVRLNNVQEGKQIETLVQILEDLLVFTYSEHASKLFGQ
KNIHVPLLIIVLDSYMRVASVQQVGWSSLCKLIEVCPGTMQSLMGPQDV
GNDWEVLGVHQLILKMLTVHNASVNLVSVIGLKTLDLLTSGKITLLIL
DEESDIFMLIFDAMHSFPANDEVQKLGCKALHVLFERVSEEQLTEFVE
NKDYMILLSALTNFKDEEEIVLHVLHCLHSLAIPCNNVEVLMSGNVRC
YNIIVVEAMKAFPMSERIQEVSCCLLHRLTLGNFFNILVLNEVHEFVVK
AVQQYPENAALQISALSCLALLTETIFLNQDLEEKNEQENDDEGEED
KLFWLEACYKALTWHRKNKHVQEAACWALNNLLMYQNSLHEKIGDEDG
HFPAHREVMLSMLMHSSSKEVFQASANALSTLLEQNVNFRKILLSKGI
HLNVLELMQKHIHSPEVAESGCKMLNHLFEGSNTSLDIMAAVVPKILT
VMKRHETSLPVQLEALRAILHFIVPGMPEESREDETFHHKLNMVKKQC
FKNDIHKVLVLAALNRFIGNPGIQKCGLKVISSIVHFPDALEMLSLEGA
MDSVLHTLQMYPPDDQEIQCLGLSLIGYLITKKNVFIGTGHLLAKILVS
SLYRFKDVAEIQTKGFQTIILAILKLSASF SKLLVHHSFDLVIHFQMS
NIMEQKDQQFLNLCKCFKAVAMDDYLKNVMLERACDQNNSIMVECLL
LLGADANQAKEGSSLICQVCEKESPKLVELLNNSGSREQDVRKALTI
SIGKGD SQIISLLRRLALDVANNSICLGGFCIGKVEPSWLGPLFPDK
TSNLRKQTNIASTLARMVIRYQMKSAVEEGTAGSDGNFSEDVLSKFD
EWFIPDSSMDSVFAQSDDLSEGESEGSFLVKKKSNSISVGEFYRDAV
LQRCSPNLQRHSNSLGPFDHEDLLKRKRKILSSDDSLRSSKQLQSHMR
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.

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Protease cleavage PreScission (LEVLFGQP) residues 221 – 228

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

Nucleotide Sequence Of Insert

ggatccATGGCTAGTGACAGCTGTCAGGGGTGCGAAGAGGACGAGGAACTCTGAAGAAGTTGAT
AGTCAGGCTGAACAATGTCCAGGAAGGAAAACAGATAGAAACGCTGGTCCAAATCCTGGAGGATC
TGCTGGTGTTCACGTACTCCGAGCACGCCTCCAAGTTATTTCAAGGCAAAAATATCCATGTGCCT
CTGTTGATCGTCTTGGACTCCTATATGAGAGTCGCGAGTGTGCAGCAGGTGGTGGTCACTTCT
GTGCAAATTAATAGAAGTCTGTCCAGGTACAATGCAAAGCTTAATGGGACCCAGGATGTTGGAA
ATGATTGGGAAGTCCTTGGTGTTCACCAATTGATTCTTAAAAATGCTAACAGTTCATAATGCCAGT
GTAACCTTGTCAGTGATTGGACTGAAGACCTTAGATCTCCTCCTAACCTCAGGTAAAATCACCTT
GCTGATATTTGGATGAAGAAAGTGATATTTTCATGTTAATTTTTGATGCCATGCACTCATTTCCAG
CCAATGATGAAGTCCAGAAACTTGGATGCAAAGCTTTACATGTGCTGTTTGAGAGAGTCTCAGAG
GAGCAACTGACTGAATTTGTTGAGAACAAAGATTATATGATATTTGTTAAGTGCCTTAACAAATTT
TAAAGATGAAGAGGAAAATTGTGCTTCATGTGCTGCATTTGTTTACATTCCTAGCGATTCCTTGCA
ATAATGTGGAAGTCCTCATGAGTGGCAATGTCAGGTGTTATAATATTGTGGTGGAAAGCTATGAAA
GCATTCCTTATGAGTGAAAGAATTCAAGAAGTGAGTTGCTGTTTGCTCCATAGGCTTACATTAGG
TAATTTTTTTCAATATCCTGGTATTAACGAAGTCCATGAGTTTGTGGTGAAGCTGTGCAGCAGT
ACCCAGAGAATGCAGCATTGCAGATCTCAGCGCTCAGCTGTTTGGCCCTCCTCACTGAGACTATT
TTCTTAAATCAAGATTTAGAGGAAAAGAATGAGAATCAAGAGAATGATGATGAGGGGGAAGAAGA
TAAATTGTTTTTGGCTGGAAGCCTGTTACAAAGCATTAACTGTCATAGAAAGAACAAGCACGTGC
AGGAGGCCGCATGCTGGGCACTAAATAATCTCCTTATGTACCAAACAGTTTACATGAGAAGATT
GGAGATGAAGATGGCCATTTCCAGCTCATAGGGAAGTGATGCTCTCCATGCTGATGCATTCCTC
ATCAAAGGAAGTTTTCCAGGCATCTGCGAATGCATTGTCAACTCTCTTAGAACAAAATGTTAATT
TCAGAAAAATACTGTTATCAAAGGAATACACCTGAATGTTTTGGAGTTAATGCAGAAGCATATA
CATTCTCCTGAAGTGGCTGAAAGTGGCTGTAAAATGCTAAATCATCTTTTTGAAGGAAGCAACAC
TTCCCTGGATATAATGGCAGCAGTGGTCCCCAAAATACTAACAGTTATGAAACGTCAATGAGACAT
CATTACCAGTGCAGCTGGAGGCGCTTCGAGCTATTTTACATTTTATAGTGCCGGCATGCCAGAA
GAATCCAGGGAGGATACAGAATTTTCATCATAAGCTAAATATGGTTAAAAAACAGTGTTCAGAA
TGATATTCACAACTGGTCCTAGCAGCTTTGAACAGGTTTCATTGGAAATCCTGGGATTCAGAAAT
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GGTTTTAAGTCTTATAGGATACTTGATTACAAAGAAGAATGTGTTTCATAGGAAGTGGACATCTGC
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TTTCAGACAATCTTAGCAATCCTCAAATTTGTCAGCATCTTTTTCTAAGCTGCTGGTGCATCATT
ATTTGACTTAGTAATATTCATCAAATGTCTTCCAATATCATGGAACAAAAGGATCAACAGTTTC
TAAACCTCTGTTGCAAGTGTTTTGCAAAAGTAGCTATGGATGATTACTTAAAAAATGTGATGCTA
GAGAGAGCGTGTGATCAGAATAACAGCATCATGGTTGAATGCTTGCTTCTATTTGGGAGCAGATGC
CAATCAAGCAAAGGAGGGATCTTCTTTAATTTGTCAGGTATGTGAGAAAGAGAGCAGTCCCAAAT
TGGTGGAACTCTTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGC
ATTGGGAAAGGTGACAGCCAGATCATCAGCTTGCTCTTAAAGGAGGCTGGCCCTGGATGTGGCCAA
CAATAGCATTTGCCTTGGAGGATTTTGTATAGGAAAAGTTGAACCTTCTTGGCTTGGTCCCTTAT
TTCCAGATAAGACTTCTAATTTAAGGAAAACAAAATATAGCATCTACACTAGCAAGAATGGTG
ATCAGATATCAGATGAAAAGTGTGTGGAAGAAGGAACAGCCTCAGGCAGCGATGGAAATTTTTC
TGAAGATGTGCTGTCTAAATTTGATGAATGGACCTTTATTCTGACTCTTCTATGGACAGTGTGT
TTGCTCAAAGTGATGACCTGGATAGTGAAGGAAGTGAAGGCTCATTTCTTGTGAAAAAGAAATCT
AATTCAATTAGTGTAGGAGAATTTTACCGAGATGCCGTATTACAGCGTTGCTCACCAATTTGCA
AAGACATTCCAATTCCTTGGGGCCATTTTTGATCATGAAGATTTACTGAAGCGAAAAAGAAAA
TACTATCTTCAGATGATTCACTCAGGTTCATAAACTTCAATCCCATATGAGGCATTCAGACAGC
ATTTCTTCTCTGGCTTCTGAGAGAGAATATATTACATCACTAGACCTTTCAGCAAATGAACTAAG
AGATATTGATGCCCTAtagcggccgc

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