

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of LRRK2 [1 - 1000] C727D

Enzyme description:- LRRK2 [1 – 1000] C727D

Clone number:- DU 62050

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 138, 958.82 daltons

Average Mass 139, 048.64 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] C727D

Protein LRRK2 [1 – 1000] C727D

Clone number DU 62050

Species Human

Accession number NM_198578.3

Tags N-terminal GST

Bacterially
expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKL TQSM A I RY I ADKH NMLGGCPKERA E ISMLE
GAVLDIRYGVSRIAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN
GDHVTHPDFM LYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVL FQGPLGSMASGSCQGC
E EDEETLKKLIVRLNNVQEGKQ IETLVQILEDLLVFTYSEHASKL FQG
KNIHVPLLI VLDSYMRVASVQQVGSLLCKLIEVCPGTMQSLMGPQDV
GNDWEVLGVHQLILKMLTVHNASVNL SVIGLKTLDLLTSGKITLLIL
DEESDIFMLIFDAMHSFPANDEVQKLGCKALHVL FERVSEEQLTEFVE
NKDYMILLSALT NFKDEE EIVLHV LHCLHSLAIPCNNVEVLM SGNVRC
YNIVVEAMKAFPMSERIQEVSCLLHRLTLGNFFN I LVLNEVHEFVVK
AVQQYPENAALQISALSCLALLTETIFLNQDLEEK NENQENDDEGEED
KLFWLEACYKALTWHRKNKHVQEAACWALNNLLMYQNSLHEKIGDEDG
HFPAHREVMLSMLMHSSSKEVFQASANALSTLLEQNVNFRKILLSKGI
HLNVLELMQKHIHSPEVAESGCKMLNHLFEGSNTSLD IMAAVVPKILT
VMKRHETSLPVQLEALRAILHFIVPGMPEESREDFH HKLNMVKKQC
FKNDIHKLVLAALNRFIGNPGIQKCGLKVISSIVHF PDALEMLSLEGA
MDSVLHTLQMYPPDQEIQCLGLSLIGYLITKKNVFIGTG HLLAKILVS
SLYRFKDVAEIQTKGFQ TILAILKLSASF SKLLVHHSFDLVI FHQMS
NIMEQKDQQFLNLCKCFAKVAMDDYLKNVMLERACDQNS IMVE**DLL**
LLGADANQAKEGSSLICQVCEKESP KLV ELLN SSGSREQDVRKALTI
SIGKGDSQIISLLLRRRLALDVANNSICLGGFCIGKVEPSWLGPLFPDK
TSNLRKQTNIAS TLARMVIRYQMKSAVEEGTASGSDGNFSE DVL SKFD
EWTFIPDSSMDSVFAQSDDL DSEGSEGSFLVKKKSNSISVGEFYRDAV
LQRCSPNLQRHSNSLGP I FDHEDLLKRKRKILSSDDSLRSSK LQSHMR
HSDSISSLASEREYITSLDLSANELRDI DAL

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 C727D mutation. Residue C727 is equivalent to D958 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

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ggatccATGGCTAGTGGCAGCTGTCAGGGGTGCGAAGAGGACGAGGAACTCTGAAGAAGTTGATAGTCAGGCTGAAC
AATGTCCAGGAAGGAAAACAGATAGAAAACGCTGGTCCAAATCCTGGAGGATCTGCTGGTGTTCACGTACTCCGAGCAC
GCCTCCAAGTTATTTCAAGGCCAAAATATCCATGTGCCTCTGTTGATCGTCTTGGACTCCTATATGAGAGTCCGCGAGT
GTGCAGCAGGTGGGTTGGTCACTTCTGTGCAAATTAATAGAACTGTGTCCAGGTACAATGCAAAGCTTAATGGGACCC
CAGGATGTTGGAAATGATTGGGAAGTCTTGGTGTTCACCAATTGATTCTTAAAAATGCTAACAGTTTCATAATGCCAGT
GTAAACTTGTGTCAGTGATTGGACTGAAGACCTTAGATCTCCTCCTAACTTCAGGTAATAATCACCTTGCTGATATTGGAT
GAAGAAAGTGATATTTTCATGTTAATTTTTGATGCCATGCACTCATTTCAGCCAATGATGAAGTCCAGAAACTTGGA
TGCAAAGCTTTACATGTGCTGTTTGAGAGAGTCTCAGAGGAGCAACTGACTGAATTTGTTGAGAACAAAGATTATATG
ATATTGTTAAGTGCCTAACAAATTTTAAAGATGAAGAGGAAATTTGTGCTTCATGTGCTGCATTGTTTACATTCCCTA
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GCATTCCCTATGAGTGAAGAATTCAGAAGTGAAGTGTGCTGTTTGTCCATAGGCTTACATTAGGTAATTTTTTCAAT
ATCCTGGTATTAACGAAGTCCATGAGTTTGTGGTGAAGCTGTGCAGCAGTACCCAGAGAATGCAGCATTGCAGATC
TCAGCGCTCAGCTGTTTGGCCCTCCTCACTGAGACTATTTTCTTAAATCAAGATTTAGAGGAAAAGAATGAGAATCAA
GAGAATGATGATGAGGGGGAAGAAGATAAAATTTGTTTTGGCTGGAAAGCTGTTACAAAGCATTAACGTGGCATAGAAAAG
AACAAAGCACGTGCAGGAGGCCGCATGCTGGGCACTAAATAATCTCCTTATGTACCAAAACAGTTTACATGAGAAGATT
GGAGATGAAGATGGCCATTTCCAGCTCATAGGGAAGTGATGCTCTCCATGCTGATGCATTCTTCATCAAAGGAAGTT
TTCCAGGCATCTGCGAATGCATTGTCAACTCTCTTAGAACAAAATGTTAATTTTCAGAAAAATACTGTTATCAAAGGA
ATACACCTGAATGTTTTGGAGTTAATGCAGAAGCATATACATTCTCCTGAAGTGGCTGAAAGTGGCTGTAAAATGCTA
AATCATCTTTTTGAAGGAAGCAACTTCCCTGGATATAATGGCAGCAGTGGTCCCCAAAATACTAACAGTTATGAAA
CGTCATGAGACATCATTACCAGTGCAGCTGGAGGCGCTTCGAGCTATTTTACATTTTATAGTGCCTGGCATGCCAGAA
GAATCCAGGGAGGATACAGAATTTTCATCATAAGCTAAATATGGTTAAAAAACAGTGTTTCAAGAATGATATTCACAAA
CTGGTCTTAGCAGCTTTGAACAGGTTCAATTGGAAATCCTGGGATTCAGAAAATGTGGATTAAAAAGTAATTTCTTCTATT
GTACATTTTCTGATGCATTAGAGATGTTATCCCTGGAAGGTGCTATGGATTTCAGTGCTTCACACACTGCAGATGTAT
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TTTCAGACAATCTTAGCAATCCTCAAATTTGTCAGCATCTTTTTCTAAGCTGCTGGTGCATCATTCAATTTGACTTAGTA
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AAAGTAGCTATGGATGATTACTTAAAAAATGTGATGCTAGAGAGAGCGTGTGATCAGAATAACAGCATCATGGTTGAA
GACTTGCTTCTATTGGGAGCAGATGCCAATCAAGCAAAGGAGGGATCTTCTTAAATTTGTCAGGTATGTGAGAAAGAG
AGCAGTCCCAAATTTGGTGGAACTCTTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGC
ATTGGGAAAGGTGACAGCCAGATCATCAGCTTGCTCTTAAAGGAGGCTGGCCCTGGATGTGGCCAACAATAGCATTTCG
CTTGGAGGATTTTGTATAGGAAAAGTTGAACCTTCTTGGCTTGGTCTTTTATTTCCAGATAAGACTTCTAATTTAAGG
AAACAAACAAATATAGCATCTACACTAGCAAGAATGGTGTATCAGATATCAGATGAAAAGTGTGTGGAAGAAGGAACA
GCCTCAGGCAGCGATGGAAAATTTTCTGAAGATGTGCTGTCTAAATTTGATGAATGGACCTTTATTCCTGACTCTTCT
ATGGACAGTGTGTTTGGCTCAAAGTGTGACCTGGATAGTGAAGGAAGTGAAGGCTCATTCTTGTGAAAAAGAAATCT
AATTCAATTAGTGTAGGAGAATTTTACCGAGATGCCGTATTACAGCGTTGCTCACCAAATTTGCAAAGACATTCCAAT
TCCTTGGGGCCCATTTTTGATCATGAAGATTTACTGAAGCGAAAAAGAAAAATACTATCTTCAGATGATTCACTCAGG
TCATCAAACTTCAATCCCATATGAGGCATTTCAGACAGCATTTCTTCTCTGGCTTCTGAGAGAGAATATATTACATCA
CTAGACCTTTCAGCAAATGAACTAAGAGATATTGATGCCCTAtagcgccgc
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