

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of LRRK2 [1 - 1000] M712V

Enzyme description:- LRRK2 [1 – 1000] M712V

Clone number:- DU 27968

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 138, 914.83 daltons

Average Mass 139, 004.63 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] M712V

Protein LRRK2 [1 – 1000] M712V

Clone number DU 27968

Species Human

Accession number NM_198578.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKL TQSM A I RY I ADKHNMLGGCPKERAE ISMLE
GAVLDIRYGVSRIAYS KDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSMASGSCQGC
EDEETLKKLIVRLNNVQEGKQIETLVQILEDLLVFTYSEHASKLFOG
KNIHVPLLI VLDSYMRVASVQQVGWSSLCKLIEVCPGTMQSLMGPQDV
GNDWEVLGVHQLILKMLTVHNASVNLSVIGLKTLDLLLTSGKITLLIL
DEESDIFMLIFDAMHSFPANDEVQKLGCKALHVLFERVSEEQLTEFVE
NKDYMILLSALTNFKDEEEIVLHVLHCLHSLAIPCNNVEVLMSGNVRC
YNIVVEAMKAFPMSERIQEVSCLLHRLTLGNFFNILVLNEVHEFVVK
AVQQYPENAALQISALSCLALLTETIFLNQDLEEKNEQENDDEGEED
KLFWLEACYKALTWHRKNKHVQEAACWALNNLLMYQNSLHEKIGDEDG
HFPAHREVMLSMLMHSSSKEVFQASANALSTLLEQNVNFRKILLSKGI
HLNVLELMQKHIHSPEVAESGCKMLNHLFEGSNTSLDIMAAVVPKILT
VMKRHETSLPVQLEALRAILHFIVPGMPEESREDTEFHKLNMVKKQC
FKNDIHKLVLAALNRFIGNPGIQKCGLKVISSIVHFDALEMLSLEGA
MDSVLHTLQMYPDQEIQCLGLSLIGYLITKKNVFIGTGHELLAKILVS
SLYRFKDVAEIQTKGFQTI LA I LKLSASF SKLLVHHSFDLVI FHQMS
NIMEQKDQQFLNLCKCFAKVAMDDYLKNV VLERACDQNSIMVECLL
LLGADANQAKEGSSLICQVCEKESP KLV ELLNSGSREQDVRKALTI
SIGKGDSQIISLLLRRALDVANNSICLGGFCIGKVEPSWLGPLFPDK
TSNLRKQTNIASTLARMVIRYQMKSAVEEGTASGSDGNFSEDVLSKFD
EWTFIPDSSMDSVFAQSDDL DSEGSEGSFLVKKKSNSISVGEFYRDAV
LQRCSPNLQRHSNSLGPFDHEDLLKRKRKILSSDDSLRSSKLQSHMR
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 M712V mutation. Residue M712 is equivalent to V943 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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ggatccATGGCTAGTGGCAGCTGTCAGGGGTGCGAAGAGGACGAGGAAACTCTGAAGAAGTTGATAGTCAGGCTGAAC
AATGTCCAGGAAGGAAAACAGATAGAAAACGCTGGTCCAAATCCTGGAGGATCTGCTGGTGTTCACGTACTIONCCGAGCAC
GCCTCCAAGTTATTTCAAGGCAAAAATATCCATGTGCCTCTGTTGATCGTCTTGACTCCTATATGAGAGTCGCGAGT
GTGCAGCAGGTGGGTTGGTCACTTCTGTGCAAATTAATAGAAGTCTGTCCAGGTACAATGCAAAGCTTAATGGGACCC
CAGGATGTTGGAAATGATTGGGAAGTCTTGGTGTTCACCAATTGATTCTTAAAAATGCTAACAGTTCATAATGCCAGT
GTAAACTTGTGAGTATTGGACTGAAGACCTTAGATCTCCTCCTAACTTCAGGTAATAACACCTTGCTGATATTGGAT
GAAGAAAGTGATATTTTCATGTTAATTTTTGATGCCATGCACTCATTTCAGCCAATGATGAAGTCCAGAAACTTGGA
TGCAAAGCTTTACATGTGCTGTTTGAGAGAGTCTCAGAGGAGCAACTGACTGAATTTGTTGAGAACAAAGATTATATG
ATATTGTTAAGTGCCTTAACAAATTTTAAAGATGAAGAGGAAATTTGTGCTTCATGTGCTGCATTGTTTACATTCCCTA
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GCATTCCCTATGAGTGAAGAATTCAGAAGTGAAGTGGCTGTTTGTCTCCATAGGCTTACATTAGGTAATTTTTTCAAT
ATCCTGGTATTAACGAAGTCCATGAGTTTGTGGTGAAGCTGTGCAGCAGTACCCAGAGAATGCAGCATTGCAGATC
TCAGCGCTCAGCTGTTTGGCCCTCCTCACTGAGACTATTTCTTAAATCAAGATTTAGAGGAAAAGAATGAGAATCAA
GAGAATGATGATGAGGGGGAAGAATAAATTTGTTTGGCTGGAAGCCTGTTACAAAGCATTAACTGCATAGAAAAG
AACAAGCACGTGCAGGAGGCCGATGCTGGGCACTAAATAATCTCCTTATGTACCAAACAGTTTACATGAGAAGATT
GGAGATGAAGATGGCCATTTCCAGCTCATAGGGAAGTGAAGTCTCCTCATGCTGATGCATTCTTCATCAAAGGAAGTT
TTCCAGGCATCTGCGAATGCATTGTCAACTCTCTTAGAACAATAATGTTAATTTTCAAGAAAAATACTGTTATCAAAGGA
ATACACCTGAATGTTTTGGAGTGAATGCAGAAGCATATACATTCTCCTGAAGTGGCTGAAAGTGGCTGTAATAATGCTA
AATCATCTTTTTGAAGGAAGCAACACTTCCCTGGATATAATGGCAGCAGTGGTCCCCAAAATACTAACAGTTATGAAA
CGTCATGAGACATCATTACCAGTGCAGCTGGAGGCGCTTCGAGCTATTTTACATTTTATAGTGCCTGGCATGCCAGAA
GAATCCAGGGAGGATACAGAATTTTCATCATAAGCTAAATATGGTTAAAAAACAGTGTTCAGAATGATATTCACAAA
CTGGTCCCTAGCAGCTTTGAACAGGTTTATTGGAAATCCTGGGATTGAGAAATGTGGATTAAAAGTAATTTCTTCTATT
GTACATTTTCTGATGCATTAGAGATGTTATCCCTGGAAGGTGCTATGGATTTCAGTGTTCACACACTGCAGATGTAT
CCAGATGACCAAGAAATTCAGTGTCTGGGTTTAAAGTCTTATAGGATACTTGATTACAAAAGAAGAATGTGTTTCATAGGA
ACTGGACATCTGCTGGCAAAAATCTGGTTCAGCTTATAACCGATTTAAGGATGTTGCTGAAATACAGACTAAAGGA
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AAAGTAGCTATGGATGATTACTTAAAAAATGTGGTGTAGAGAGAGCGTGTGATCAGAATAACAGCATCATGGTTGAA
TGCTTGCTTCTATTGGGAGCAGATGCCAATCAAGCAAAGGAGGGATCTTCTTTAATTTGTGAGGATGTGAGAAAAGAG
AGCAGTCCCAAATTTGGTGGAACTCTTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGC
ATTGGGAAAGGTGACAGCCAGATCATCAGCTTGTCTTAAAGGAGGCTGGCCCTGGATGTGGCCAACAATAGCATTTCG
CTTGGAGGATTTTGTATAGGAAAAGTTGAACCTTCTTGGCTTGGTCTTTATTTCCAGATAAGACTTCTAATTTAAGG
AAACAAACAAATATAGCATCTACACTAGCAAGAATGGTGTATCAGATATCAGATGAAAAGTGTGTGGAAGAAGGAACA
GCCTCAGGCAGCGATGGAAATTTTTCTGAAGATGTGCTGTCTAAATTTGATGAATGGACCTTTATTCCCTGACTCTTCT
ATGGACAGTGTGTTTGTCTCAAAGTGTGACCTGGATAGTGAAGGAAGTGAAGGCTCATTTCCTGTGAAAAAGAAATCT
AATTCATTTAGTGTAGGAGAATTTTACCAGATGCCGTATTACAGCGTTGCTCACCAAATTTGCAAAGACATTCCAAT
TCCTTGGGGCCCATTTTTGATCATGAAGATTTACTGAAGCGAAAAAGAAAAATACTATCTTCAGATGATTCACACTCAGG
TCATCAAACCTTCAATCCCATATGAGGCATTGAGACAGCATTTCTTCTCTGGCTTCTGAGAGAGAATATATTACATCA
CTAGACCTTTTCAGCAAATGAACTAAGAGATATTGATGCCCTATagcggccgc
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