

# *MRC PPU REAGENTS*

## Standard Operating Procedure

### Preparation of LRRK2 [1 - 1000] L728D

**Enzyme description:-** LRRK2 [1 – 1000] L728D

**Clone number:-** DU 27220

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 138, 948.75 daltons

Average Mass 139, 038.61 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] L728D

Protein LRRK2 [1 – 1000] L728D

Clone number DU 27220

Species Human

Accession number NM\_198578.3

Tags N-terminal GST

Bacterially  
expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKL TQSM A I RY I ADKHNMLGGCPKERA E ISMLE  
GAVLDIRYGVSRIAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN  
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY  
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVL FQGPLGSMASGSCQGC  
**E EDEETLKKLIVRLNNVQEGKQ IETLVQILEDLLVFTYSEHASKL FQG**  
**KNIHVPLLI VLDSYMRVASVQQVGW SLLCKLIEVCPGTMQSLMGPQDV**  
**GNDWEVLGVHQLILKMLTVHNASVNLSVIGLKTLDLLLTSGKITLLIL**  
**DEESDIFMLIFDAMHSFPANDEVQKLGCKALHVL FERVSEEQLTEFVE**  
**NKDYMILLSALT NFKDEE EIVLHVLHCLHSLAIPCNNVEVLM SGNVRC**  
**YNIVVEAMKAFPMSERIQEVSCLLHRLTLGNFFN I LVLNEVHEFVVK**  
**AVQQYPENAALQISALSCLALLTETIFLNQDLEEK NENQENDDEGEED**  
**KLFWLEACYKALTWHRKNKHVQEAACWALNNLLMYQNSLHEKIGDEDG**  
**HFPAHREVMLSMLMHSSSKEVFQASANALSTLLEQNVNFRKILLSKGI**  
**HLNVLELMQKHIHSPEVAESGCKMLNHLFEGSNTSLD IMAAVVPKILT**  
**VMKRHETSLPVQLEALRAILHFIVPGMPEESREDETFH HKLNMVKKQC**  
**FKNDIHKLVLAALNRFIGNPGIQKCGLKVISSIVHF PDALEMLSLEGA**  
**MDSVLHTLQMYPDQEI IQCLGLSLIGYLITKKNVFIGTG HLLAKILVS**  
**SLYRFKDVAEIQTKGFQ TILAILKLSASF SKLLVHHSFDLVI FHQMS**  
**NIMEQKDQQFLNLCKCFAKVAMDDYLKNVMLERACDQNS IMVECDL**  
**LLGADANQAKEGSSLICQVCEKES SPKLV ELLN SSGSREQDVRKALTI**  
**SIGKGDSQIISLLLRR LALDVANNSICLGGFCIGKVEPSWLGPLFPDK**  
**TSNLRKQTNIASTLARMVIRYQMKSAVEEGTASGSDGNF SEDVLSKFD**  
**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 L728D mutation. Residue L728 is equivalent to D959 of the fusion protein.

## MRC PPU REAGENTS

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
AATGTCCAGGAAGGAAAACAGATAGAAAACGCTGGTCCAAATCCTGGAGGATCTGCTGGTGTTCACGTACTIONCGAGCAC
GCCTCCAAGTTATTTCAAGGCCAAAAATATCCATGTGCCTCTGTTGATCGTCTTGGACTCCTATATGAGAGTCGCGAGT
GTGCAGCAGGTGGGTTGGTCACTTCTGTGCAAATTAATAGAAGTCTGTCCAGGTACAATGCAAAGCTTAATGGGACCC
CAGGATGTTGGAAATGATTGGGAAGTCCTTGGTGTTCACCAATTGATTCTTAAAAATGCTAACAGTTCATAATGCCAGT
GTAAACTTGTGAGTATTGGACTGAAGACCTTAGATCTCCTCCTAACTTCAGGTAATAACACCTTGCTGATATTGGAT
GAAGAAAGTGATATTTTCATGTTAATTTTTGATGCCATGCACTCATTTCAGCCAATGATGAAGTCCAGAAACTTGGA
TGCAAAGCTTTACATGTGCTGTTTGGAGAGTCTCAGAGGAGCAACTGACTGAATTTGTTGAGAACAAAGATTATATG
ATATTGTTAAGTGCCTTAACAAATTTTAAAGATGAAGAGGAAATTTGTGCTTCATGTGCTGCATTGTTTACATTCCCTA
GCGATTCCCTTGAATAATGTGGAAGTCCTCATGAGTGGCAATGTCCAGGTGTTATAATATTGTGGTGGAAAGCTATGAAA
GCATTCCCTATGAGTGAAGAATTCAGAAGTGAAGTGGCTGTTTGTCTCCATAGGCTTACATTAGGTAATTTTTTCAAT
ATCCTGGTATTAACGAAGTCCATGAGTTTGTGGTGAAGCTGTGCAGCAGTACCCAGAGAATGCAGCATTGCAGATC
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GAGAATGATGATGAGGGGGAAGAATAAATTTGTTTGGCTGGAAGCCTGTTACAAAGCATTAACTGCGCATAGAAAG
AACAAGCACGTGCAGGAGGCCGCATGCTGGGCCTAAATAATCTCCTTATGTACCAAACAGTTTACATGAGAAGATT
GGAGATGAAGATGGCCATTTCCAGCTCATAGGGAAGTGAAGTCTCCTCATGCTGATGCATTCTTCATCAAAGGAAGTT
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ATACACCTGAATGTTTTGGAGTGAATGCAGAAGCATATACATTCTCCTGAAGTGGCTGAAAGTGGCTGTAATAATGCTA
AATCATCTTTTTGAAGGAAGCAACACTTCCCTGGATATAATGGCAGCAGTGGTCCCCAAAATACTAACAGTTATGAAA
CGTCATGAGACATCATTACCAGTGCAGCTGGAGGCGCTTCAGACTATTTTACATTTTATAGTGCCTGGCATGCCAGAA
GAATCCAGGGAGGATACAGAATTTTCATCATAAGCTAAATATGGTTAAAAAACAGTGTTCAGAATGATATTCACAAA
CTGGTCCCTAGCAGCTTTGAACAGGTTTCAATTGGAAATCCTGGGATTGAGAAATGTGGATTAAAAAGTAATTTCTTCTATT
GTACATTTTCTGATGCATTAGAGATGTTATCCCTGGAAGGTGCTATGGATTGAGTGTTCACACACTGCAGATGTAT
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ACTGGACATCTGCTGGCAAAAATCTGGTTCAGCTTATAACCGATTTAAGGATGTTGCTGAAATACAGACTAAAGGA
TTTTAGACAATCTTAGCAATCCTCAAATTTGTCAGCATCTTTTTCTAAGCTGCTGGTGCATCATTCAATTTGACTTAGTA
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AAAGTAGCTATGGATGATTACTTAAAAAATGTGATGCTAGAGAGAGCGTGTGATCAGAATAACAGCATCATGGTTGAA
TGCGATCTTCTATTGGGAGCAGATGCCAATCAAGCAAAGGAGGGATCTTCTTTAATTTGTGAGGATGTGAGAAAGAG
AGCAGTCCCAAATTTGGTGGAACTCTTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGC
ATTGGGAAAGGTGACAGCCAGATCATCAGCTTGTCTTAAAGGAGGCTGGCCCTGGATGTGGCCAACAATAGCATTTCG
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TCATCAAACCTTCAATCCCATATGAGGCATTGAGACAGCATTCTTCTCTGGCTTCTGAGAGAGAATATATTACATCA
CTAGACCTTTTCAGCAAATGAACTAAGAGATATTGATGCCCTATagcgggccgc
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