MRC PPU REAGENTS

Standard Operating Procedure

Preparation of LRRK2 [1 - 1000] L728D L729D

Enzyme description:-	LRRK2 [1 – 1000] L728D L729D
Clone number:-	DU 27189
Source:-	Recombinant
Expression system:-	*E.coli*
Tag:-	N-terminal GST
Purification method:-	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	138, 950.69 daltons
Average Mass	139, 040.54 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	5.47
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

MRC PPU REAGENTS

Clone Data Sheet

LRRK2 [1 – 1000] L728D L729D

Protein	LRRK2 [1 – 1000] L728D L729D
Clone number	DU 27189
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** **NIMEQKDQQFLNLCCKCFAKVAMDDYLKNVMLERACDQNNSIMVECDD** **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 L728D L729D mutation. Residue L728 is equivalent to D959 and L729 is equivalent to D960 of the fusion protein.

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
TGCGATGACCTATTGGGAGCAGATGCCAATCAAGCAAAGGAGGGATCTTCTTTAATTTGTCAGGTATGTGAGAAAGAG
AGCAGTCCCAAATTGGTGGAACTCTTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGC
ATTGGGAAAGGTGACAGCCAGATCATCAGCTTGCTCTTAAGGAGGCTGGCCCTGGATGTGGCCAACAATAGCATTTGC
CTTGGAGGATTTTGTATAGGAAAAGTTGAACCTTCTTGGCTTGGTCCTTTATTTCCAGATAAGACTTCTAATTTAAGG
AAACAAACAAATATAGCATCTACACTAGCAAGAATGGTGATCAGATATCAGATGAAAAGTGCTGTGGAAGAAGGAACA
GCCTCAGGCAGCGATGGAAATTTTTCTGAAGATGTGCTGTCTAAATTTGATGAATGGACCTTTATTCCTGACTCTTCT
ATGGACAGTGTGTTTGCTCAAAGTGATGACCTGGATAGTGAAGGAAGTGAAGGCTCATTTCTTGTGAAAAAGAAATCT
AATTCAATTAGTGTAGGAGAATTTTACCGAGATGCCGTATTACAGCGTTGCTCACCAAATTTGCAAAGACATTCCAAT
TCCTTGGGGCCCATTTTTGATCATGAAGATTTACTGAAGCGAAAAAGAAAAATACTATCTTCAGATGATTCACTCAGG
TCATCAAAACTTCAATCCCATATGAGGCATTCAGACAGCATTTCTTCTCTGGCTTCTGAGAGAGAATATATTACATCA
CTAGACCTTTCAGCAAATGAACTAAGAGATATTGATGCCCTAtagcggccgc
```