Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of LRRK2tide / Nictide

Enzyme description:- LRRK2tide / Nictide

Clone number:- DU 17157

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 29, 555.14 daltons
Average Mass 29, 574.33 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8.19

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

LRRK2tide / Nictide

Protein	LRRK2tide / Nictide
Clone number	DU 17157
Species	Artificial
Accession number	NA
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**RLGWWRFYT LRRARQGNTKQR**
Native sequence	Amino acids R1 – R21 (end) of the LRRK2 substrate peptide. Residue R232 of fusion protein is equivalent to R1 of the LRRK2 substrate peptide The GST tag is located at residues 1 – 220.
Protease cleavage	Prescission site (LEVLFQGP) at residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 site of pGex6P-1
Nucleotide sequence	ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCC ACTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTG TATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTG GGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAA TTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAA GGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGT AAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAA ATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAAT GGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTA GTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTAC TTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCC ACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGGAAGTTCTG TTCCAGGGGCCCCTGGGATCCAGACTAGGTTGGTGGAGATTTTATACA CTACGACGGGCCAGGCAGGGCAATACAAAGCAGAGAtag