

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of MOESIN [1 - 577]

Protein description:- MOESIN [1 - 577]

Clone number:- DU 10618

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 95,562.95 daltons

Average Mass 95,623.31 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.02

Purity:- > 85 %

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 °C

Assay:- Substrate for LRRK2

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CLONE DATA SHEET

MOESIN [1 - 577]

<u>Protein</u>	MOESIN [1 - 577]
<u>Clone number</u>	DU 10618
<u>Species</u>	Human
<u>Accession number</u>	NM_002444.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAIEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFGPLGSPGIPGSTR AAMPKTISVRVTMDAELEFAIQPNTTGKQLFDQVVKTIGLREVWFFG LQYQDTKGFSTWLKLNKKVTAQDVRKESPLLFFKFRKFYPEDVSEELI QDITQRLFFLQVKEGILNDDIYCPPETAVLLASYAVQSKYGDFNKEVH KSGYLAGDKLLPQRVLEQHKLNKDQWEERIQVWHEEHRGMLREDAVLE YLKIAQDLEMYGVNYFSIKNKKGSELWLGVDAALGLNIYEQNDRLTPKI GFPWSEIRNISFNDKKFVIKPIDKKAPDFVFYAPRLRINKRILALCMG NHELYMRRRKPDITIEVQQMKQAQAREEKHQKQMERAMLENEKKKREMAE KEKEKIEREKEELMERLKQIEEQTKKAQQELEEQTRRALELEQERKRA QSEAEKLAKERQEAEEAKEALLQASRDQKKTQEQLALEMAELTARISQ LEMARQKKESEAVEWQQAQMVQEDLEKTRAEKLTAMSTPHVAEPAEN EQDEQDENGAEASADLRADAMAKDRSEEERTTEAEKNERVQKHLKALT SELANARDESKKTANDMIHAENMRLGRDKYKTLRQIRQGNTKQRIDEF ESM
<u>Native sequence</u>	Amino acids M1 – M577 of human MOESIN. Residue M243 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (LEVLFGQP) residues 221 - 228

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Cloning sites

NotI and *NotI* sites of pGEX-6P

Nucleotide sequence of insert

GCGGCCGCCATGCCCAAACGATCAGTGTGCGTGTGACCACCATGGAT
GCAGAGCTGGAGTTTGCCATCCAGCCCAACACCACCGGGAAGCAGCTA
TTTGACCAGGTGGTGAAAACCTATTGGCTTGAGGGAAGTTTGGTTCTTT
GGTCTGCAGTACCAGGACACTAAAGGTTTCTCCACCTGGCTGAAACTC
AATAAGAAGGTGACTGCCCAGGATGTGCGGAAGGAAAGCCCCCTGCTC
TTTAAGTTCCGTGCCAAGTTCTACCCTGAGGATGTGTCCGAGGAATTG
ATTCAGGACATCACTCAGCGCCTGTTCTTTCTGCAAGTGAAAGAGGGC
ATTCTCAATGATGATATTTACTGCCCGCTGAGACCGCTGTGCTGCTG
GCCTCGTATGCTGTCCAGTCTAAGTATGGCGACTTCAATAAGGAAGTG
CATAAGTCTGGCTACCTGGCCGGAGACAAGTTGCTCCCGCAGAGAGTC
CTGGAACAGCACAACTCAACAAGGACCAGTGGGAGGAGCGGATCCAG
GTGTGGCATGAGGAACACCGTGGCATGCTCAGGGAGGATGCTGTCCTG
GAATATCTGAAGATTGCTCAAGATCTGGAGATGTATGGTGTGAACTAC
TTCAGCATCAAGAACAAGAAAGGCTCAGAGCTGTGGCTGGGGGTGGAT
GCCCTGGGTCTCAACATCTATGAGCAGAATGACAGACTAACTCCCAAG
ATAGGCTTCCCCTGGAGTGAAATCAGGAACATCTCTTTCAATGATAAG
AAATTTGTCATCAAGCCCATTGACAAAAAAGCCCCGGACTTCGTCTTC
TATGCTCCCCGGCTGCGGATTAACAAGCGGATCTTGGCCTTGTGCATG
GGGAACCATGAACTATACATGCGCCGTCGCAAGCCTGATACCATTGAG
GTGCAGCAGATGAAGGCACAGGCCCGGGAGGAGAAGCACCAGAAGCAG
ATGGAGCGTGCTATGCTGGAAAATGAGAAGAAGAAGCGTGAAATGGCA
GAGAAGGAGAAAGAGAAGATTGAACGGGAGAAGGAGGAGCTGATGGAG
AGGCTGAAGCAGATCGAGGAACAGACTAAGAAGGCTCAGCAAGAAGTG
GAAGAACAGACCCGTAGGGCTCTGGAACCTTGAGCAGGAACGGAAGCGT
GCCCAGAGCGAGGCTGAAAAGCTGGCCAAGGAGCGTCAAGAAGCTGAA
GAGGCCAAGGAGGCCTTGCTGCAGGCCTCCCGGGACCAGAAAAAGACT
CAGGAACAGCTGGCCTTGGAATGGCAGAGCTGACAGCTCGAATCTCC
CAGCTGGAGATGGCCCGACAGAAGAAGGAGAGTGAGGCTGTGGAGTGG
CAGCAGAAGGCCCAGATGGTACAGGAAGACTTGAGAGAAGACCCGTGCT
GAGCTGAAGACTGCCATGAGTACACCTCATGTGGCAGAGCCTGCTGAG
AATGAGCAGGATGAGCAGGATGAGAATGGGGCAGAGGCTAGTGCTGAC
CTACGGGCTGATGCTATGGCCAAGGACCGCAGTGAGGAGGAACGTACC
ACTGAGGCAGAGAAGAATGAGCGTGTGCAGAAGCACCTGAAGGCCCTC
ACTTCGGAGCTGGCCAATGCCAGAGATGAGTCCAAGAAGACTGCCAAT
GACATGATCCATGCTGAGAACATGCGACTGGGCCGAGACAAATACAAG
ACCCTGCGCCAGATCCGGCAGGGCAACACCAAGCAGCGCATTGACGAA
TTTGAGTCTATGtaagcggccgc