

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of EZRIN [1 - 586]

Protein description:- EZRIN [1 - 586]

Clone number:- DU 10788

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 97,018.74 daltons

Average Mass 97,079.94 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.86

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

EZRIN [1 - 586]

Protein EZRIN [1 - 586]

Clone number DU 10788

Species Human

Accession number BC068458

Tags N-terminal GST

Bacterially expressed protein MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMGGCPKERAIEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSPEFALMHTS
MPKPINVRVTMTDAELEFAIQPNTTGKQLFDQVVKTIGLREVWYFGLH
YVDNKGFPWTWLKLDKKVSAQEVVRKENPLQFKFRAKFYPEDVAEELIQD
ITQKLFFLQVKEGILSDEIYCPPETAVLLGSYAVQAKFGDYNKEVHKS
GYLSSERLIPQRVMDQHKLTRDQWEDRIQVWHAHRGMLKDNAMLEYL
KIAQDLEMYGINYFEIKNKKGTDLWLGVDALGLNIYEKDDKLTPIGF
PWSEIRNISFNDKKFVIKPIDKKAPDFVYAPRLRINKRILQLCMGNH
ELYMRRRKPDTEVQOMKAQAREEKHQKQLERQOLETEKKRRETVERE
KEQMMREKEELMLRLQDYEEKTKKAERELSEQIQRALQLEEERKRAQE
EAERLEADRMAALRAKEELERQAVDQIKSQEQLAELA EYTAKIALLE
EARRRKEDEVEEWQHRAKEAQDDL VKTKEELHLVMTAPPPPPPPVYEP
VSYHVQESLQDEGA EPTGYSAGLSSEGI RDDGNEEKRITEAEKNERVQ
RQLLTLSSSELSQARDENKRTHNDI IHNENMRQGRDKYKTLRQIRQGNT
KQRIDEFEAL

Native sequence Amino acids M1 – L586 of human EZRIN.
Residue M241 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

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Cloning sites

*Spe*I and *Not*I sites of pGEX-6P

Nucleotide sequence of insert

ggatcccCGGAATTGCGCCTTATGCATACTAGTATGCCGAAACCAATC
AATGTCCGAGTTACCACCATGGATGCAGAGCTGGAGTTTGCAATCCAG
CCAAATACAACCTGGAAAACAGCTTTTTTGATCAGGTGGTAAAGACTATC
GGCCTCCGGGAAGTGTGGTACTTTGGCCTCCACTATGTGGATAATAAA
GGATTTTCCTACCTGGCTGAAGCTGGATAAGAAGGTGTCTGCCCAGGAG
GTCAGGAAGGAGAATCCCCTCCAGTTCAAGTTCCGGGCCAAGTTCTAC
CCTGAAGATGTGGCTGAGGAGCTCATCCAGGACATCACCCAGAAACTT
TTCTTCCCTCCAAGTGAAGGAAGGAATCCTTAGCGATGAGATCTACTGC
CCCCCTGAGACTGCCGTGCTCTTGGGGTCCTACGCTGTGCAGGCCAAG
TTTGGGGACTACAACAAAGAAGTGCACAAGTCTGGGTACCTCAGCTCT
GAGCGGCTGATCCCTCAAAGAGTGATGGACCAGCACAACTTACCAGG
GACCAGTGGGAGGACCGGATCCAGGTGTGGCATGCGGAACACCGTGGG
ATGCTCAAAGATAATGCTATGTTGGAATACCTGAAGATTGCTCAGGAC
CTGGAAATGTATGGAATCAACTATTTTCGAGATAAAAAACAAGAAAGGA
ACAGACCTTTGGCTTGGAGTTGATGCCCTTGGACTGAATATTTATGAG
AAAGATGATAAGTTAACCCCAAAGATTGGCTTTCCTTGGAGTGAAATC
AGGAACATCTCTTCAATGACAAAAAGTTTGTCAATAAACCCATCGAC
AAGAAGGCACCTGACTTTGTGTTTTATGCCCCACGTCTGAGAATCAAC
AAGCGGATCCTGCAGCTCTGCATGGGCAACCATGAGTTGTATATGCGC
CGCAGGAAGCCTGACACCATCGAGGTGCAGCAGATGAAGGCCCAGGCC
CGGGAGGAGAAGCATCAGAAGCAGCTGGAGCGGCAACAGCTGGAAACA
GAGAAGAAAAGGAGAGAAACCGTGGAGAGAGAGAAAGAGCAGATGATG
CGCGAGAAGGAGGAGTTGATGCTGCGGCTGCAGGACTATGAGGAGAAG
ACAAAGAAGGCAGAGAGAGAGCTCTCGGAGCAGATTGAGAGGGCCCTG
CAGCTGGAGGAGGAGAGGAAGCGGGCACAGGAGGAGGCCGAGCGCCTA
GAGGCTGACCGTATGGCTGCACTGCGGGCTAAGGAGGAGCTGGAGAGA
CAGGCGGTGGATCAGATAAAGAGCCAGGAGCAGCTGGCTGCGGAGCTT
GCAGAATACACTGCCAAGATTGCCCTCCTGGAAGAGGCGCGGAGGCGC
AAGGAGGATGAAGTTGAAGAGTGGCAGCACAGGGCCAAAGAAGCCAG
GATGACCTGGTGAAGACCAAGGAGGAGCTGCACCTGGTGATGACAGCA
CCCCCGCCCCCACCACCCCCCGTGTACGAGCCGGTGAGCTACCATGTC
CAGGAGAGCTTGCAGGATGAGGGCGCAGAGCCCACGGGCTACAGCGCG
GGGCTGTCTAGTGAGGGCATCCGGGATGACGGCAATGAGGAGAAGCGC
ATCACTGAGGCAGAGAAGAACGAGCGTGTGCAGCGGCAGCTGCTGACG
CTGAGCAGCGAGCTGTCCCAGGCCCGAGATGAGAATAAGAGGACCCAC
AATGACATCATCCACAACGAGAACATGAGGCAAGGCCGGGACAAGTAC
AAGACGCTGCGGCAGATCCGGCAGGGCAACACCAAGCAGCGCATCGAC
GAGTTCGAGGCCCTGtaagcggccgc