Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of RADIXIN [1 - 583]

Protein description:- Radixin [1 - 583]

Clone number:- DU 10790

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 95, 328.97 daltons
Average Mass 95, 389.01daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.84

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

RADIXIN [1 - 583]

Protein	Radixin [1 - 583]
Clone number	DU 10790
Species	Human
Accession number	NM_002906.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MPKPINVRV TTMDAELEFAIQPNTTGKQLFDQVVKTVGLREVWFFGLQYVDSKGYST WLKLNKKVTQQDVKKENPLQFKFRAKFFPEDVSEELIQEITQRLFFLQ VKEAILNDEIYCPPETAVLLASYAVQAKYGDYNKEIHKPGYLANDRLL PQRVLEQHKLTKEQWEERIQNWHEEHRGMLREDSMMEYLKIAQDLEMY GVNYFEIKNKKGTELWLGVDALGLNIYEHDDKLTPKIGFPWSEIRNIS FNDKKFVIKPIDKKAPDFVFYAPRLRINKRILALCMGNHELYMRRRKP DTIEVQQMKAQAREEKHQKQLERAQLENEKEKREIAEKEKERIEREKE ELMERLKQIEEQTIKAQKELEEQTRKALELDQERKRAKEEAERLEKER RAAEEAKSAIAKQAADQMKNQEQLAAELAEFTAKIALLEEAKKKKEEE ATEWQHKAFAAQEDLEKTKEELKTVMSAPPPPPPPPVIPPTENEHDEH DENNAEASAELSNEGVMNHRSEEERVTETQKNERVKKQLQALSSELAQ ARDETKKTQNDVLHAENVKAGRDKYKTLRQIRQGNTKQRIDEFEAM**
Native sequence	Amino acids M1 – M583 of human Radixin. Residue M232 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

Cloning sites

*Bam*HI and *Not*I sites of pGEX-6P-1

Nucleotide sequence of insert

ggatccATGCCGAAACCAATCAACGTAAGAGTAACTACAATGGATGCT
GAGCTGGAATTTGCCATTCAGCCCAATACAACTGGCAAACAACTTTTT
GACCAGGTGGTGAAAACAGTTGGTTTGCGTGAGGTCTGGTTTTTTGGG
CTGCAGTATGTAGACAGCAAAGGTTATTCTACATGGCTTAAACTAAAT
AAAAAGGTAACACAGCAGGATGTTAAAAAAGAGAATCCTTTACAGTTC
AAGTTTAGAGCTAAATTCTTTCCTGAAGATGTTTCTGAGGAATTAATT
CAAGAAATAACCCAGAGACTCTTCTTCTTGCAAGTTAAAGAAGCCATC
TTAAATGATGAGATATATTGCCCGCCAGAAACTGCAGTTCTTTTGGCT
TCCTATGCTGTCCAAGCCAAGTATGGAGATTACAATAAAGAGATTCAT
AAGCCAGGCTACCTGGCTAATGATAGACTCCTACCCCAGCGTGTATTG
GAACAACACAAACTAACAAAAGAACAGTGGGAAGAAAGAATACAGAAC
TGGCATGAAGAACATAGAGGAATGTTAAGGGAGGATTCTATGATGGAA
TACCTGAAGATTGCACAAGATCTAGAAATGTATGGAGTCAACTATTTT
GAAATAAAAAATAAAAAAGGAACTGAATTGTGGCTAGGTGTTGATGCT
TTGGGTCTGAATATTTATGAGCATGACGACAAGTTAACACCTAAAATT
GGTTTTCCCTGGAGTGAAATCAGAAATATTTCATTTAATGACAAAAAA
TTTGTTATAAAGCCAATCGACAAAAAGGCACCTGATTTTGTGTTTTAT
GCACCTCGTCTGAGAATCAATAAGAGGATTTTGGCCTTATGTATGGGA
AACCATGAACTATACATGCGAAGAAGGAAGCCTGATACTATTGAAGTA
CAACAGATGAAGGCTCAGGCTAGGGAGGAGAAACATCAGAAGCAGTTG
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GAACAGACTCGAAAAGCTCTAGAACTGGATCAAGAACGAAAACGAGCA
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GCAAAGTCTGCCATAGCAAAACAAGCTGCCGACCAGATGAAGAATCAG
GAGCAGCTAGCAGCAGAACTTGCTGAATTCACTGCCAAGATTGCACTT
CTAGAGGAAGCCAAGAAGAAAAAGGAAGAGGAAGCTACTGAGTGGCAA
CACAAAGCTTTTGCAGCCCAGGAAGACTTGGAAAAGACCAAAGAAGAG
TTAAAAACTGTGATGTCTGCCCCCCCTCCACCTCCACCACCACCAGTC
ATTCCTCCAACAGAAAACGAACATGATGAACACGATGAGAATAATGCT
GAAGCTAGTGCTGAATTATCAAATGAAGGGGTAATGAACCATAGAAGC
GAGGAAGAACGTGTAACCGAAACACAGAAAAATGAGCGTGTTAAGAAG
CAACTTCAGGCATTAAGTTCAGAATTAGCCCAAGCCAGAGATGAAACC
AAGAAAACACAAAATGATGTTCTTCATGCTGAGAATGTTAAAGCAGGC
CGTGATAAGTACAAGACTCTGCGACAGATTCGACAAGGCAATACAAAG
CAGCGTATCGATGAGTTTGAAGCAATGTGAgcggccgc