

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Sharpin isoform b

Enzyme description:- Sharpin isoform b 1- 326 (full length)

Clone number:- DU32976

Source:- Recombinant

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Expression level:- 1 mg/L

Calculated molecular mass:-

Monoisotopic 60663 Da

Average Mass 60698 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.95

Purity:- 50 %

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM TCEP

Storage temperature:- -80°C

Assay:-

Division of Signal Transduction Therapy

Clone Data Sheet

Protein name GST-Sharpin isoform b

Protein Sharpin isoform b 1-326 (full length)

Synonyms

Clone Number DU32976

Species Human

Accession Number Protein: AAH25244.1

Tag N-terminal GST

Aminoacid sequence of the expressed protein .

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLE
FPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAELISMLEGAVLDI
RYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF
MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQ
GWQATFGGGDHPPKSDLEVLFOGPLGSMAPPAGGAAAAASDLGSAAVLLAV
HAAVRPLGAGPDAAEQLRRLQLSADPERPGRFRLELLGAGPGAVNLEWPLE
SVSYTIRGPTQHELQPPPGGPGTLSLHFLNPOEAQRWAVLVRGATVEGONG
SKSNSPPALGPEACPVSLSPPPEASTLKGPPPEADLPRSPGNLTEREELAG
SLARAIAGGDEKGAQAQVAVLAQHRVALSVQLQEQACFPFGPIRLQVTLEDA
ASAASAASSAHVALQVHPHCTVAALQEQVFSLEGFPAVQRWVIGRCLCVP
ERSLASYGVRQDGP AFLYLLSAPREAPAQLVLSFLHLHQCPRPWL

Native sequence in bold

Protease cleavage Precission Protease site underlined

Cloning sites BamH1 / NotI

DNA sequence of insert

GGATCCATGGCGCCGAGCGGGCGGGCGGGCGGGCGGGCCTCGGACTTG
GGCTCCGCCGAGTGCTCTTGCTGTGCACGCCGCGGTGAGGCCGCTGGGC
GCCGGCCAGACGCCGAGGCACAGCTGCGGAGGCTGCAGCTGAGCGCGGAC
CCTGAGAGGCCTGGGCGCTTCCGGCTGGAGCTGCTGGGCGCGGGACCTGGG
GCGGTAAATTTGGAGTGGCCCCCTGGAGTCAGTTTCCTACACCATCCGAGGC
CCCACCCAGCACGAGCTACAGCTCCACCAGGAGGGCCTGGAACCCTCAGC
CTGCACTTCCTCAACCCTCAGGAAGCTCAGCGGTGGGCGAGTCCTAGTCCGA
GGTGCCACCGTGGAAGGACAGAAATGGCAGCAAGAGCAACTCACCACCAGCC
TTGGGCCCAGAAGCATGCCCTGTCTCCCTGCCAGTCCCCCGGAAGCCTCC
ACACTCAAGGGCCCTCCACCTGAGGCAGATCTTCCTAGGAGCCCTGGAAAC
TTGACGGAGAGAGAAGAGCTGGCAGGGAGCCTGGCCCCGGGCTATTGCAGGT
GGAGACGAGAAGGGGGCAGCCCAAGTGGCAGCCGTCTGGCCCAGCATCGT
GTGGCCCTGAGTGTTCAGCTTCAGGAGGCTGCTTCCACCTGGCCCCATC
AGGCTGCAGGTACACTTGAAGACGCTGCCTCTGCCGCATCCGCCGCGTCC
TCTGCACACGTTGCCCTGCAGGTCCACCCCCACTGCACTGTTGCAGCTCTC
CAGGAGCAGGTGTTCTCAGAGCTCGGTTTCCCGCCAGCCGTGCAACGCTGG
GTCATCGGACGGTGCCTGTGTGTGCCTGAGCGCAGCCTTGCCTCTTACGGG
GTTCCGGCAGGATGGGGACCCTGCTTTCCTCTACTTGCTGTCAGCTCCTCGA
GAAGCCCCAGCCAGCTGGTCCCTGTCTTCCCTGCACCTTCATCAATGCCCC
AGACCGCCCTGGCTGTGAGCGGCCGC