Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-USP27x

Enzyme description:- GST-USP27x

Clone number:- SC21193

Source:- BL21 Recombinant

Tag:- N-terminal GST tag

Purification method:- GSH-sepharose, SEC

Expression level:- 0.5 mg/L

Calculated molecular mass:-

Monoisotopic	76404 Da
Average Mass	76453 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.81

Purity:- 80%

Enzyme storage buffer:-

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

Storage temperature:- -80ºC

Assay:-

Ub-Rho110-Gly cleavage assay monitored by Ex/Em 485/535 nm

Assay buffer:-

40 mM Tris pH 7.5, 100 mM NaCl, 5 mM DTT, 0.01% Triton X-100, 0.005% Ovalbumin, 0.5 μM Ub-Rho110-Gly

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Clone Data Sheet

GST-USP27x

Protein	GST-USP27x
Synonyms	UBP27x, USP22L
Clone Number	SC21193
Species	Human
Accession Number	Protein: A6NNY8.3 DNA: NM_001145073.1
Tags	N-terminal GST tag
Amino acid sequence of expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MCKDYVYDKDIEQIAKEEQ GEALKLQASTSTEVSHQQCSVPGLGEKFPTWETTKPELELLGHNPRRRRI TSSFTIGLRGLINLGNTCFMNCIVQALTHTPILRDFFLSDRHRCEMPSPE LCLVCEMSSLFRELYSGNPSPHVPYKLLHLVWIHARHLAGYRQQDAHEFL IAALDVLHRHCKGDDVGKAANNPNHCNCIIDQIFTGGLQSDVTCQACHGV STTIDPCWDISLDLPGSCTSFWPMSPGRESSVNGESHIPGITTLTDCLRR FTRPEHLGSSAKIKCGSCQSYQESTKQLTMNKLPVVACFHFKRFEHSAKQ RRKITTYISFPLELDMTPFMASSKESRMNGQLQLPTNSGNNENKYSLFAV VNHQGTLESGHYTSFIRHHKDQWFKCDDAVITKASIKDVLDSEGYLLFYH KQVLEHESEKVKEMNTQAY**
Native sequence	in bold
Protease cleavage	Precission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

GGATCCATGTGTAAGGACTATGTATATGACAAAGACATTGAGCAAATTGCCAA
AGAAGAGCAAGGAGAAGCTTTGAAATTACAAGCCTCCACCTCAACAGAGGTTT
CTCACCAGCAGTGTTCAGTGCCAGGCCTTGGTGAGAAATTCCCAACCTGGGAA
ACAACCAAACCAGAATTAGAACTGCTGGGGCACAACCCGAGGAGAAGAAGAAT
CACCTCCAGCTTTACGATCGGTTTAAGAGGACTCATCAATCTTGGCAACACGT
GCTTTATGAACTGCATTGTCCAGGCCCTCACCCACACGCCGATACTGAGAGAT
TTCTTTCTCTCTGACAGGCACCGATGTGAGATGCCGAGTCCCGAGTTGTGTCT
GGTCTGTGAGATGTCGTCGCTGTTTCGGGAGTTGTATTCTGGAAACCCGTCTC
CTCATGTGCCCTATAAGTTACTGCACCTGGTGTGGATACATGCCCGCCATTTA
GCAGGGTACAGGCAACAGGATGCCCACGAGTTCCTCATTGCAGCGTTAGATGT
CCTGCACAGGCACTGCAAAGGTGATGATGTCGGGAAGGCGGCCAACAATCCCA
ACCACTGTAACTGCATCATAGACCAAATCTTCACAGGTGGCCTGCAGTCTGAT
GTCACCTGTCAAGCCTGCCATGGCGTCTCCACCACGATAGACCCATGCTGGGA
CATTAGTTTGGACTTGCCTGGCTCTTGCACCTCCTTCTGGCCCATGAGCCCAG
GGAGGGAGAGCAGTGTGAACGGGGAAAGCCACATACCAGGAATCACCACCCTC
ACGGACTGCTTGCGGAGGTTTACGAGGCCAGAGCACTTAGGAAGCAGTGCCAA
AATCAAATGTGGTAGTTGCCAAAGCTACCAGGAATCTACCAAACAGCTCACAA
TGAATAAATTACCTGTCGTTGCCTGTTTTCATTTCAAACGGTTTGAACATTCA
GCGAAACAGAGGCGCAAGATCACTACATACATTTCCTTTCCTCTGGAGCTGGA
TATGACGCCGTTTATGGCCTCAAGTAAAGAGAGCAGAATGAATGGACAATTGC
AGCTGCCAACCAATAGTGGAAACAACGAAAATAAGTATTCCTTGTTTGCTGTG
GTTAATCACCAAGGAACCTTGGAGAGTGGCCACTATACCAGCTTCATCCGGCA
CCACAAGGACCAGTGGTTCAAGTGTGATGATGCCGTCATCACTAAGGCCAGTA
TTAAGGACGTACTGGACAGTGAAGGGTATTTACTGTTCTATCACAAACAGGTG
CTAGAACATGAGTCAGAAAAAGTGAAAGAAATGAACACACAAGCCTACTGAGC
GGCCGC