Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-USP18

Enzyme description:- GST-USP18

Clone number:- DU4925

Source:- BL21 Recombinant

Tag:- N-terminal GST tag

Purification method:- GSH-Sepharose

Expression level:- 1 mg/L

Calculated molecular mass:-

Monoisotopic	69789 Da
Average Mass	69883 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.3

Purity:- 30%

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-USP18

Protein	GST-USP18
Synonyms	hUBP43
Clone Number	DU4925
Species	Human
Accession Number	Protein: Q9UMW8 DNA: NM_017414.3
	N-terminal GST tag
Amino acid sequence of expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSKAFGLLRQICQSILAES SQSPADLEEKKEEDSNMKREQPRERPRAWDYPHGLVGLHNIGQTCCLNSL IQVFVMNVDFTRILKRITVPRGADEQRRSVPFQMLLLLEKMQDSRQKAVR PLELAYCLQKCNVPLFVQHDAAQLYLKLWNLIKDQITDVHLVERLQALYT IRVKDSLICVDCAMESSRNSSMLTLPLSLFDVDSKPLKTLEDALHCFFQP RELSSKSKCFCENCGKKTRGKQVLKLTHLPQTLTIHLMRFSIRNSQTRKI CHSLYFPQSLDFSQILPMKRESCDAEEQSGGQYELFAVIAHVGMADSGHY CVYIRNAVDGKWFCFNDSNICLVSWEDIQCTYGNPNYHWQETAYLLVYMK MEC**
Native sequence	in bold
Protease cleavage	Precission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

GGATCCATGAGCAAGGCGTTTGGGCTCCTGAGGCAAATCTGTCAGTCCATCCT
GGCTGAGTCCTCGCAGTCCCCGGCAGATCTTGAAGAAAAGAAGGAAGAAGACA
GCAACATGAAGAGAGAGCAGCCCAGAGAGCGTCCCAGGGCCTGGGACTACCCT
CATGGCCTGGTTGGTTTACACAACATTGGACAGACCTGCTGCCTTAACTCCTT
GATTCAGGTGTTCGTAATGAATGTGGACTTCACCAGGATATTGAAGAGGATCA
CGGTGCCCAGGGGAGCTGACGAGCAGAGGAGAAGCGTCCCTTTCCAGATGCTT
CTGCTGCTGGAGAAGATGCAGGACAGCCGGCAGAAAGCAGTGCGGCCCCTGGA
GCTGGCCTACTGCCTGCAGAAGTGCAACGTGCCCTTGTTTGTCCAACATGATG
CTGCCCAACTGTACCTCAAACTCTGGAACCTGATTAAGGACCAGATCACTGAT
GTGCACTTGGTGGAGAGACTGCAGGCCCTGTATACGATCCGGGTGAAGGACTC
CTTGATTTGCGTTGACTGTGCCATGGAGAGTAGCAGAAACAGCAGCATGCTCA
CCCTCCCACTTTCTCTTTTTGATGTGGACTCAAAGCCCCTGAAGACACTGGAG
GACGCCCTGCACTGCTTCTTCCAGCCCAGGGAGTTATCAAGCAAAAGCAAGTG
CTTCTGTGAGAACTGTGGGAAGAAGACCCGTGGGAAACAGGTCTTGAAGCTGA
CCCATTTGCCCCAGACCCTGACAATCCACCTCATGCGATTCTCCATCAGGAAT
TCACAGACGAGAAAGATCTGCCACTCCCTGTACTTCCCCCAGAGCTTGGATTT
CAGCCAGATCCTTCCAATGAAGCGAGAGTCTTGTGATGCTGAGGAGCAGTCTG
GAGGGCAGTATGAGCTTTTTGCTGTGATTGCGCACGTGGGAATGGCAGACTCC
GGTCATTACTGTGTCTACATCCGGAATGCTGTGGATGGAAAATGGTTCTGCTT
CAATGACTCCAATATTTGCTTGGTGTCCTGGGAAGACATCCAGTGTACCTACG
GAAATCCTAACTACCACTGGCAGGAAACTGCATATCTTCTGGTTTACATGAAG
ATGGAGTGCTAAGCGGCCGC