Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-OTU1

Enzyme description:- GST-OTU1

Clone number:- DU36559

Source:- BL21 Recombinant

Tag:- N-terminal

Purification method:- GSH sepharose

Expression level:- 4 mg/L

Calculated molecular mass:-

Monoisotopic	65104 Da
Average Mass	65144 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.70

Purity:- 90%

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-OTU1

Protein	GST-OTU1
Synonyms	YOD1, DUBA-8, OTUD2, Hin-7
Clone Number	DU36559
Species	Human
Accession Number	Protein: Q5VVQ6 DNA: NM018566.3
Tags	N-terminal GST tag
Amino acid sequence of expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFP NLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGV SRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDAL DVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGG GDHPPKSDLEVLFQGPLGS**MFGPAKGRHFGVHPAPGFPGGVSQQAAGTKAGPA** **GAWPVGSRTDTMWRLRCKAKDGTHVLQGLSSRTRVRELQGQIAAITGIAPGGQ** **RILVGYPPECLDLSNGDTILEDLPIQSGDMLIIEEDQTRPRSSPAFTKRGASS** **YVRETLPVLTRTVVPADNSCLFTSVYYVVEGGVLNPACAPEMRRLIAQIVASD** **PDFYSEAILGKTNQEYCDWIKRDDTWGGAIEISILSKFYQCEICVVDTQTVRI** **DRFGEDAGYTKRVLLIYDGIHYDPLQRNFPDPDTPPLTIFSSNDDIVLVQALE** **LADEARRRRQFTDVNRFTLRCMVCQKGLTGQAEAREHAKETGHTNFGEV**
Native sequence	in bold
Protease cleavage	Precission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

GGATCCATGTTTGGCCCCGCTAAAGGTCGCCATTTTGGAGTCCACCCGGCGCC
TGGTTTCCCCGGCGGCGTCTCCCAACAGGCTGCCGGGACCAAAGCTGGCCCCG
CGGGTGCCTGGCCTGTGGGCAGCCGGACCGACACGATGTGGCGGCTCCGCTGC
AAGGCCAAGGACGGCACCCATGTTTTGCAGGGGCTGTCCAGCCGGACCCGGGT
GCGGGAACTCCAGGGCCAAATTGCCGCCATCACCGGGATCGCCCCCGGCGGTC
AGCGAATCCTCGTCGGATACCCTCCCGAGTGCCTGGATCTCAGCAATGGGGAT
ACCATTCTGGAAGACTTGCCCATCCAATCTGGTGACATGCTGATCATTGAAGA
AGACCAAACCAGGCCCAGAAGTTCACCTGCATTTACTAAACGTGGTGCTTCTA
GTTACGTCAGGGAAACTTTGCCTGTGCTTACCAGAACCGTGGTCCCAGCAGAC
AACTCTTGCCTCTTTACTAGTGTGTACTATGTCGTCGAAGGAGGAGTCTTGAA
TCCAGCTTGTGCCCCTGAGATGAGACGCCTCATAGCACAAATTGTAGCAAGCG
ATCCAGACTTCTATAGTGAGGCAATACTGGGAAAAACAAATCAAGAGTACTGT
GACTGGATCAAAAGGGATGACACTTGGGGAGGAGCAATAGAGATATCGATTTT
GTCCAAGTTTTACCAATGTGAAATATGTGTAGTGGATACACAGACAGTAAGAA
TTGATCGTTTTGGGGAAGATGCAGGATATACCAAAAGGGTTCTGCTTATTTAT
GATGGCATCCACTATGATCCACTTCAGCGTAACTTCCCTGATCCAGATACACC
TCCTCTGACCATTTTCTCCTCTAATGATGATATTGTTCTTGTACAAGCACTGG
AATTAGCAGATGAAGCTAGAAGAAGGAGACAGTTTACTGATGTCAACCGCTTC
ACCCTGAGATGCATGGTATGTCAGAAAGGATTAACTGGACAAGCAGAAGCAAG
GGAACATGCCAAGGAGACAGGCCATACCAACTTTGGAGAAGTGTGAGCGGCCG
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