Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-USP2b

Enzyme description:- USP2b

Clone number:- DU13025

Source:- BL21 Recombinant

Tag:- GST

Purification method:- GSH-Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic	72686 Da
Average Mass	72731 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.50

Purity:- 50%

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-USP2b

Protein	USP2b
Synonyms	
Clone Number	DU13025
Species	Human
Accession Number	Protein: NP_741994 DNA: NM_171997
Tags	N-terminal GST tag
Amino acid sequence of expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEF PNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRY GVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLY DALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQA TFGGGDHPPKSDLEVLFQGPLGSPNSRVD**MRTSYTVTLPEDPPAAPFPALAK ELRPRSPLSPSLLLSTFVGLLLNKAKNSKSAQGLAGLRNLGNTCFMNSILQC LSNTRELRDYCLQRLYMRDLHHGSNAHTALVEEFAKLIQTIWTSSPNDVVSP SEFKTQIQRYAPRFVGYNQQDAQEFLRFLLDGLHNEVNRVTLRPKSNPENLD HLPDDEKGRQMWRKYLEREDSRIGDLFVGQLKSSLTCTDCGYCSTVFDPFWD LSLPIAKRGYPEVTLMDCMRLFTKEDVLDGDEKPTCCRCRGRKRCIKKFSIQ RFPKILVLHLKRFSESRIRTSKLTTFVNFPLRDLDLREFASENTNHAVYNLY AVSNHSGTTMGGHYTAYCRSPGTGEWHTFNDSSVTPMSSSQVRTSDAYLLFY ELASPPSRM**
Native sequence	in bold. This is a splice variant of USP2, encompassing the catalytic domain.
Protease cleavage	Precission site underlined
Cloning sites	Sal1 / Not1

DNA sequence of insert

GGATCCCCGAATTCCCGGGTCGACATGCGCACCTCGTACACCGTGACCCTGC
CCGAGGACCCCCCCGCCGCCCCCTTTCCCGCCCTCGCCAAGGAGCTGCGGCC
GCGCTCCCCTCTCTCCCCGTCCCTGCTGCTCTCCACCTTCGTGGGGCTCCTG
CTCAACAAAGCCAAGAATTCTAAGAGTGCCCAGGGTCTGGCTGGTCTTCGAA
ACCTTGGGAACACGTGCTTCATGAACTCAATTCTGCAGTGCCTGAGCAACAC
TCGGGAGTTGAGAGATTACTGCCTCCAGAGGCTCTACATGCGGGACCTGCAC
CACGGCAGCAATGCACACACAGCCCTCGTGGAAGAGTTTGCAAAACTAATTC
AGACCATATGGACTTCATCCCCCAATGATGTGGTGAGCCCATCTGAGTTCAA
GACCCAGATCCAGAGATATGCACCGCGCTTTGTTGGCTATAATCAGCAGGAT
GCTCAGGAGTTCCTTCGCTTTCTTCTGGATGGGCTCCATAACGAGGTGAACC
GAGTGACACTGAGACCTAAGTCCAACCCTGAGAACCTCGATCATCTTCCTGA
TGACGAGAAAGGCCGACAGATGTGGAGAAAATATCTAGAACGGGAAGACAGT
AGGATCGGGGATCTCTTTGTTGGGCAGCTAAAGAGCTCGCTGACGTGTACAG
ATTGTGGTTACTGTTCTACGGTCTTCGACCCCTTCTGGGACCTCTCACTGCC
CATTGCTAAGCGAGGTTATCCTGAGGTGACATTAATGGACTGCATGAGGCTC
TTCACCAAAGAGGATGTGCTTGATGGAGATGAAAAGCCAACATGCTGTCGCT
GCCGAGGCAGAAAACGGTGTATAAAGAAGTTCTCCATCCAGAGGTTCCCAAA
GATCTTGGTGCTCCATCTGAAGCGGTTCTCAGAATCCAGGATCCGAACCAGC
AAGCTCACAACATTTGTGAACTTCCCCCTAAGAGACCTGGACTTAAGAGAAT
TTGCCTCAGAAAACACCAACCATGCTGTTTACAACCTGTACGCTGTGTCCAA
TCACTCCGGAACCACCATGGGTGGCCACTATACAGCCTACTGTCGCAGTCCA
GGGACAGGAGAATGGCACACTTTCAACGACTCCAGCGTCACTCCCATGTCCT
CCAGCCAAGTGCGCACCAGCGACGCCTACCTGCTCTTCTACGAACTGGCCAG
CCCGCCCTCCCGAATGTAGCGGCCGC