Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of USP9x [1553 – 1995]

Enzyme description:- USP9x [1553 – 1995]

Clone number:- DU20628

Source:- BL21 Recombinant

Tag:- N-terminal GST tag

Purification method:- GSH-Sepharose

Expression level:- 0.2 mg/L

Calculated molecular mass:-

Monoisotopic	78735 Da
Average Mass	78783 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.35

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

Division of Signal Transduction Therapy

Clone Data Sheet

GST-USP9x [1553 – 1995]

Protein	USP9x [1553-1995]
Synonyms	FAF-X
Clone Number	DU20628
Species	Human
Accession Number	Protein: Q93008 DNA: NM_001039591
Tags	GST
Amino acid sequence of expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFQGPLGSLEVLFQGP**KGFVGLKNAGA** **TCYMNSVIQQLYMIPSIRNGILAIEGTGSDVDDDMSGDEKQDNESNVDPR** **DDVFGYPQQFEDKPALSKTEDRKEYNIGVLRHLQVIFGHLAASRLQYYVP** **RGFWKQFRLWGEPVNLREQHDALEFFNSLVDSLDEALKALGHPAMLSKVL** **GGSFADQKICQGCPHRYECEESFTTLNVDIRNHQNLLDSLEQYVKGDLLE** **GANAYHCEKCNKKVDTVKRLLIKKLPPVLAIQLKRFDYDWERECAIKFND** **YFEFPRELDMEPYTVAGVAKLEGDNVNPESQLIQQSEQSESETAGSTKYR** **LVGVLVHSGQASGGHYYSYIIQRNGGDGERNRWYKFDDGDVTECKMDDDE** **EMKNQCFGGEYMGEVFDHMMKRMSYRRQKRWWNAYILFYERMDTIDQDDE** **LIRYISELAITTRPHQIIMPSAIERSVRKQN**
Native sequence	in bold
Protease cleavage	Precission sites underlined
Cloning sites	BamH1 /Not1

DNA sequence of insert

GGATCCCTGGAAGTTCTGTTCCAGGGCCCGAAAGGATTCGTGGGGCTGAAAA
ATGCCGGTGCTACTTGTTACATGAATTCTGTGATTCAGCAACTCTACATGAT
TCCTTCCATTAGGAACGGTATTCTTGCCATTGAAGGCACAGGTAGTGATGTA
GATGATGATATGTCTGGGGATGAGAAGCAGGACAATGAGAGCAATGTTGATC
CCAGGGATGATGTATTTGGATATCCTCAACAATTTGAAGATAAACCAGCATT
AAGTAAAACTGAAGATAGAAAAGAGTACAACATTGGTGTCCTAAGACACCTT
CAGGTCATCTTTGGTCATTTAGCTGCTTCTCGACTGCAATACTATGTGCCCA
GAGGATTTTGGAAACAGTTCAGGCTTTGGGGTGAGCCTGTTAATCTGCGTGA
ACAACACGATGCTTTAGAATTTTTTAATTCATTGGTGGATAGTTTAGATGAA
GCTTTAAAAGCTTTAGGACATCCAGCTATGCTAAGTAAAGTCTTAGGAGGTT
CCTTTGCTGATCAGAAGATCTGCCAAGGCTGCCCACATAGGTACGAATGTGA
AGAATCTTTTACGACCCTAAACGTAGACATTAGAAATCACCAAAATCTTCTT
GATTCTTTGGAACAGTATGTCAAAGGAGATTTACTAGAAGGTGCAAATGCAT
ATCATTGTGAAAAATGCAATAAAAAGGTTGATACCGTAAAGCGCTTGCTGAT
TAAAAAATTACCTCCTGTTCTTGCTATACAACTAAAGCGATTTGACTATGAC
TGGGAAAGAGAATGTGCAATCAAGTTCAATGATTATTTTGAATTTCCTCGAG
AGCTGGACATGGAACCTTACACAGTTGCAGGTGTCGCAAAGCTGGAAGGGGA
TAATGTAAACCCAGAGAGTCAGTTGATACAACAGAGTGAGCAGTCTGAAAGT
GAGACAGCAGGAAGCACAAAATACAGACTTGTGGGTGTGCTCGTACACAGTG
GTCAAGCGAGTGGGGGGCATTATTATTCTTACATCATCCAAAGGAATGGTGG
AGATGGTGAGAGAAATCGCTGGTATAAATTTGATGATGGTGATGTAACAGAA
TGTAAAATGGATGATGACGAAGAAATGAAAAACCAGTGTTTTGGTGGAGAGT
ACATGGGAGAAGTGTTTGATCACATGATGAAGCGTATGTCATACAGGCGCCA
GAAAAGGTGGTGGAATGCTTATATACTTTTTTATGAACGAATGGACACAATA
GACCAAGATGATGAGTTGATAAGATATATATCAGAGCTTGCTATCACCACCA
GACCTCATCAGATTATTATGCCATCAGCCATTGAGAGAAGTGTACGGAAACA
GAACTAGGCGGCCGC