

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-USP20

Enzyme description:- GST-USP20 1-913

Clone number:- DU12807

Source:- BL21 Recombinant

Tag:- GST

Purification method:- GSH-Sepharose

Expression level:- 0.5 mg/L

Calculated molecular mass:-

Monoisotopic 128629 Da

Average Mass 128708 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.88

Purity:- 40%

Enzyme storage buffer:-

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

Storage temperature:- -80°C

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

USP20 1-913

<u>Protein</u>	GST-USP20
<u>Synonyms</u>	VHL-interacting deubiquitinating enzyme 2, UBP20
<u>Clone Number</u>	DU12807
<u>Species</u>	Human
<u>Accession Number</u>	Protein: AAH39593 (This version lacks a D at position 359 compared to Q9Y2K6.2)
<u>Tags</u>	N-terminal GST tag
<u>Amino acid sequence of expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA WPLQGWQATFGGGDHPKSDLEVLVFOGPLGSMGDSRDLCPHLDSIGEVTK EDLLKSKGTCQSCGVTGPNLWACLQVACPVVGCGESFADHSTIHAQAKK HNLTVNLTTFRLWCYACEKEVFLEQRLAAPLLGSSSKFSEQDSPPSHPL KAVPIA VADEGESESEDDDLKPRGLTGMKNLGNSCYMNAALQALSNC PPL TQFFLECGGLVVRTDKK PALCKSYQKLVSEVWHKKRPSYVVP TSLSHGIKL VNPMFRGYAQQDTQEFRLCLMDQLHEELKEPVVATVALTEARDS DSSD TD EKREGDRSPSEDEF LSCDSSSDRGE G DGQGRGGSSQAETELLIPDEAGR AISEKERMKDRKFSWGQQRTNSEQVDE DADVD TAMAALDQPAEAQPPSPR SSSPCRTPEPDNDAHLRSSSRPCSPVHHHEGHAKLSSSPPRASPVRMAPS YVLKKAQVLSAGSRRRKEQRYRSVISDFDGSILSLVQCLTCDRVSTTVE TFQDLSLPIPGKEDLAKLHSAIYQNVPAKPGACGDSYAAQGWLAFIVEYI RRFVVSCTPSWFWGPVVTLEDCLAAFFAADELKGDNMYS CERCKLRNGV KYCKVLRRLPEILCIHLKRFRHEVMYSFKINSHVSFPLEGLDLRPFLAKEC TSQITTYDLLSVICHHG TAGSGHYIAYCQNVINGQWYEFDDQYVTEVHET VVQNAEGYVLFYRKSSEAMRERQVVSLAAMREPSLLRFYVSREWLNKF NTFAEPGPITNQTF LCSHG GIPPHKYHYIDDLVVILPQNVWEHLYNRF GG GPAVNHLVCSICQVEIEALAKRRRIEIDTFIKLNKAFQAEESPGVIYCI SMQWFREWEAFVKGKDNEPPGPIDNSRIAQVKGSGHVQLKQGADYGOISE ETWTYLNSLYGGGPEIAIRQSV AQPLG PENLHGEQKIEAETRAV
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	Precision site underlined
<u>Cloning sites</u>	BamH1 / Not1

DNA sequence of insert

GGATCCATGGGGGACTCCAGGGACCTTTGCCCTCACCTTGACTCCATAGG
AGAGGTGACCAAAAGAGGACTTGCTGCTCAAATCTAAGGGAACCTGTCAGT
CGTGTGGGGTCACCGGACCAAACCTATGGGCTGCTGTCAGGTTGCCCTGC
CCCTATGTTGGCTGCGGAGAATCCTTCGCTGACCACAGCACCATTTCATGC
ACAGGCAAAAAAGCACAACCTTGACCGTGAACCTGACCACGTTCCGACTGT
GGTGTACGCCTGTGAGAAGGAGGTATTCCTGGAGCAGCGGCTGGCAGCC
CCTCTGCTGGGCTCCTCTTCCAAGTTCTCTGAACAGGACTCCCCGCCACC
CTCCCACCCTCTGAAAGCTGTTCCCTATGCTGTGGCTGATGAAGGAGAGT
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GGCCGC