

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-USP28

<u>Enzyme description:-</u>	GST-USP28
<u>Clone number:-</u>	SC20233
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal GST tag
<u>Purification method:-</u>	GSH sepharose, SEC
<u>Expression level:-</u>	1 mg/L

Calculated molecular mass:-

Monoisotopic	149220 Da
Average Mass	149310 Da
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 5.13

Purity:- 60%

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

GST-USP28

<u>Protein</u>	GST-USP28
<u>Synonyms</u>	
<u>Clone Number</u>	SC20233
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q96RU2 DNA: BC065928.1
<u>Tags</u>	N-terminal GST tag
<u>Amino acid sequence of expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEHLIERDEGDKWRNKKFELGLEFP NLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGV SRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDAL DVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLOGWQATFGG GDHPPKSDLEVLFOGPLGSMTAELQODDAAGAADGHGSSCOMLLNQLREITGI QDPSFLHEALKASNGDITQAVSLLTDERVKEPSQDTVATEPSEVEGSAANKEV LAKVIDLTHDNKDDLQAAIALSLLSPKIQADGRDLNRMHEATS AETKRKRK RCEVWGENPNPNDWRRVDGWPVGLKNVGNTCWFSAVIQSLFQLPEFRRLVLSY SLPQNVLENCRSHTEKRNIMFMQELQYLFALMMGSNRKFVDP SAALDLLKGAF RSSEEQQQDVSEFTHKLLDWLEDAFQLAVNVNSPRNKSENPMVQLFYGTFLTE GVREGKPFCCNETFGQYPLQVNGYRNLDECLEGAMVEGDVELLPSDHSVYKYGQ ERWFTKLPPVLT FELSRFEFNQSLGQPEKIH NKLEFPQI IYMDRYMYRSKELI RNKRECIRKLKEEIKILOQKLERVVKYSGPARFPLPDMLKYVIEFASTKPAS ESCPPESDTHMTLPLSSVHCSVSDQTSKESTSTESSSQDVESTFSSPEDSLPK SKPLTSSRSSMEMPSQPAPRTVTDEEINFVKTCLQRWRSEIEQDIQDLKTCIA STQTIEQMYCDPLLROVPYRLHAVLVHEGQANAGHYWAYIYNQPRQSWLKYN DISVTESSWEEVERDSYGGLRNV SAYCLMYINDKLPYFNAEAAPTESDQMSEV EALSVELKHYIQEDNWRFEQEVEEWEQESKIPOMESSTNSSSQDYSTSQEP SVASSHGVRCLSSSEHAVIVKEQTAQA IANTARAYEKSGVEAALSEVMLSPAMQ GVILAI AKARQTFDRDGSEAGLIKAFHEEYSRLYQLAKETPTSHSDPRLQHV L VYFFQNEAPKRVERTLLEQFADKNLSYDERSISIMKVAQAKLKEIGPDDMMN EEYKWHEDYSLFRKVSVYLLTGLELYQKGYQEALS YLVYAYQSNAALLMKG PRRGVKESVIALYRRKCLELNAKAASLFETNDDHSVTEGINVMNELIIPC I H LIINNDISKDDLDAIEVMRNHWCSYLGQDIAENLQLCLGEFLPRLLDPSAEI I VLKEPPTIRPNSPYDLCSRFAAVMESIQGVSTVTVK</p>
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	Precision site underlined
<u>Cloning sites</u>	BamH1 / Not1

DNA sequence of insert

ATGACTGCGGAGCTGCAGCAGGACGACGCGGCCGGCGCGGCAGACGGCCA
CGGCTCGAGCTGCCAAATGCTGTTAAATCAACTGAGAGAAATCACAGGCA
TTCAGGACCCCTTCTTTCTCCATGAAGCTCTGAAGGCCAGTAATGGTGAC
ATTACTCAGGCAGTCAGCCTTCTCACTGATGAGAGAGTTAAGGAGCCAG
TCAAGACACTGTTGCTACAGAACCATCTGAAGTAGAGGGGAGTGCTGCCA
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AATTCAGGGAGTTTCAACTGTGACAGTGAATAA