

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP34 [1892 – 2241]

Enzyme description:- USP34 [1892 - 2241]

Clone number:- DU 61205

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 67, 420.14 daltons

Average Mass 67, 464.46 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.77

Purity:- 80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT

Storage temperature:- -70 °C

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Clone Data Sheet

USP34 [1892 - 2241]

<u>Protein</u>	USP34 [1892 - 2241]
<u>Clone number</u>	DU 61205
<u>Species</u>	Human
<u>Accession number</u>	Q70CQ2-1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSRFVGLTNLGATCYL ASTIQQLYMIPEARQAVFTAKYSEDMKHKTTLLELQKMFTYLMESECKA YNPRPFCKTYTMDKQPLNTGEQKDMTEFFTDLITKIEEMSP ELKNTVKS LFGGVITNNVSLDCEHVSQTAE EFTVRCQVADMKNIYESLDEVTIKD TLEGDNMYTCSHC GKKVRAEK RACFKKLPRILSFNTMRYTFNMVTMMKE KVNTHFSFPLRLDMTPYTEDFLMGKSERKEGFKEVSDH SKDSESYEYDL IGVTVHTGTADGGHYYSFIRDIVNPHAYKNNKWYLFNDAEVKPFDSAQL ASECFGGEMTTKTYDSVTDKFMDFSF EKTHSAYMLFYKRMEP</p>
<u>Native sequence</u>	<p>Amino acids R1891 – P2241 (end residue is S3546) of human USP34. Residue R232 of the fusion protein is equivalent to R1892 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX 6P-1

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Nucleotide
Sequence of insert

ggatccAGATTTGTTGGCCTTACTAACCTTGGAGCTACTTGTACTTAG
CTTCTACTATTTCAGCAACTTTATATGATACCTGAGGCAAGACAGGCTGT
CTTCACTGCCAAGTATTCAGAGGATATGAAGCACAAGACCACTCTTCTG
GAGCTTCAGAAAATGTTTACATATTTAATGGAGAGTGAATGCAAAGCAT
ATAATCCTAGACCTTTCTGTAAAACATACCCATGGATAAGCAGCCTCT
GAATACTGGGGAACAGAAAGATATGACAGAGTTTTTTTACTGATCTAATT
ACCAAAATCGAAGAAATGTCTCCCGAACTGAAAAATACCGTCAAAGTT
TATTTGGAGGTGTAATTACAAACAATGTTGTATCCTTGGATTGTGAACA
TGTTAGTCAAACGCTGAAGAGTTTTATACTGTGAGGTGCCAAGTGGCT
GATATGAAGAACATTTATGAATCTCTTGATGAAGTTACTATAAAAGACA
CTTTGGAAGGTGATAACATGTATACTTGTCTCATTGTGGGAAGAAAGT
ACGAGCTGAAAAAAGGGCATGTTTTAAGAAATTGCCTCGCATTTTGAGT
TTCAATACTATGAGATACACATTTAATATGGTCACGATGATGAAAGAGA
AAGTGAATACACACTTTTCCTTCCCATTACGTTTGGACATGACGCCCTA
TACAGAAGATTTTCTTATGGGAAAGAGTGAGAGGAAAGAAGGTTTTAAA
GAAGTCAGTGATCATTCAAAGACTCAGAGAGCTATGAATATGACTTGA
TAGGAGTGACTGTTACACACAGGAACGGCAGATGGTGGACACTATTATAG
CTTTATCAGAGATATAGTAAATCCCATGCTTATAAAAAACAATAAATGG
TATCTTTTTAATGATGCTGAGGTAAAACCTTTTGATTCTGCTCAACTTG
CATCTGAATGTTTTGGTGGAGAGATGACGACCAAGACCTATGATTCTGT
TACAGATAAATTTATGGACTTCTCTTTTGAAAAGACACACAGTGCATAT
ATGCTGTTTTACAAACGCATGGAACCAaagcggccgc