

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP37 [1 - 979]

<u>Enzyme description:-</u>	USP37 [1 - 979]
<u>Clone number:-</u>	DU 67226
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 137, 678.18 daltons
Average Mass 137, 764.66 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.84

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

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

Storage temperature:- -70 °C

Division of Signal Transduction Therapy

Clone Data Sheet

USP37 [1 - 979]

Protein USP37 [1 - 979]
Clone number DU 67226
Species Human
Accession number Q86T82-1
Tags N-terminal GST

**Baculovirus
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNK
KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE
RAEISMLEGAVLDIRYGVSRAYSKDFETLKVDFLSKLPEMLKM
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD
LEVLFGQPLGSPEFPGR**MSPLKIHGPIRIRSMQTGITKWEKS**
FEIVEKENKVS**LVVHYNTGGIPRIFQLSHNIKNVVLRPSGAKQS**
RLMLTLQDNSFLS**IDKVPSKDAEEMRLFLDAVHQNRLPAAMKPS**
QSGSFGAILGSR**TSQKETSRLSYSDNQASAKRGSLETKDDIP**
FRKVLGNPGRGSIKT**VAGSGIARTIPSLTSTSTPLRSGLLENRT**
EKRKRMI**STGSELNEDYPKENDSSSNKAMTDPSRKYLTSREK**
QLSLKQSEENRTSGLLPLQSSSFYGS**RAGSKEHSSGGTNLDRTN**
VSSQTPSAKRSLGFLPQP**VPLSVKLCRCNQDYTGWNKPRVPLSS**
HQQQLOQF**SNLGNTCYMNAILOSLFSLQSFANDLLKQGI****PWKK**
IPLNALIRRF**AHLLVKKDCNSETKKDLLKVKNAISATAERFS**
GYMQND**AHEFLSQCLDQKEDMEKLNKTWKTEPVS****GEENSPDIS**
ATRAYTCPVITNLEFEVQHSI**ICKACGEIIPKREQFN****DLSIDLP**
RRKKPLPPRSIQD**SLDLFFRAEELEYSCEKCGGKCALVRHKFNR**
LPRVLI**LHLKRYSFNVALSLN****NIKIGQOVIIPRYLTLSS****HCTENT**
KPPFTL**GWSAHMAISRPLKASQMVNSCITSPSTPSKKFTFKSKS**
SLALCLDSDSE**DELKRSVALSQR****LCEMLGNEQQQEDLEKDSKLC**
PIEPDKSE**LENSGFDRMSEELLA****AVLEISKRDASPSLSHEDDD**
KPTSSPDTGFAEDDIQ**EMPENPDTMETEKPKTITELDPAS****FTEI**
TKDCDEN**KENKTPEGSQGEVDWLQOYD****MEREREEQELQQALAQS**
LQEQAWEQKEDDLK**RATELSLQEFNNSFVDALGSDEDSG****NED**
VFDMEY**TEAEAEELKRNAETGNLPHSYRLISV****VSHIGSTSSSGH**
YISDVYDIKKQAWFTYNDLE**VSKIQEAAVQSDRDRSGYIFFYMH**
KEIFDELLETEKNSQSLSTEVGKTTRQAS

Native sequence

Amino acids M1 – S979 (end residue) of human USP37.
Residue M239 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.

Division of Signal Transduction Therapy

The following amino acid substitution is present:

L – S, where L979 of the native enzyme is S1217 of the fusion protein.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *SalI*+2 and *NotI* sites of pFastBac Dual.

Division of Signal Transduction Therapy

Nucleotide sequence of insert

cgactcATGTCTCCTCTGAAGATACATGGTCCTATCAGAATTTCGAAGTA
TGCAGACTGGGATTACAAAGTGGAAAGAAGGATCCTTTGAAATTGTAGA
AAAAGAGAATAAAGTCAGCCTAGTAGTTCCTACTACAATACTGGAGGAATT
CCAAGGATATTTTCAGCTAAGTCATAACATTAATAAATGTGGTGCTTCGAC
CCAGTGGAGCGAAACAAAGCCGCCTAATGTAACTCTGCAAGATAACAG
CTTCTTGTCTATTGACAAAGTACCAAGTAAGGATGCAGAGGAAATGAGG
TTGTTTCTAGATGCAGTCCATCAAAACAGACTTCTGCAGCCATGAAAC
CGTCTCAGGGGTCTGGTAGTTTTTGGAGCCATTCTGGGCAGCAGGACCTC
ACAGAAGGAAACCAGCAGGCAGCTTTCTTACTCAGACAATCAGGCTTCT
GCAAAAAGAGGAAGTTTGGAAACTAAAGATGATATTCATTTTCGAAAAG
TTCTTGGTAATCCGGGTAGAGGATCGATTAAGACTGTAGCAGGAAGTGG
AATAGCTCGGACGATTCCTTCTTTGACATCTACTTCAACACCTCTTAGA
TCAGGGTTGCTAGAAAATCGTACTGAAAAGAGGAAAAGAATGATATCAA
CTGGCTCAGAATTGAATGAAGATTACCCTAAGGAAAATGATTTCATCATC
GAACAACAAGGCCATGACAGATCCCTCCAGAAAGTATTTAACCAGCAGT
AGAGAAAAGCAGCTGAGTTTGAACAGTCAGAAGAGAATAGGACATCAG
GGCTTTTACCTTTACAGTCATCATCTTTTATGGTAGCAGAGCTGGATC
CAAGGAACACTCTTCTGGTGGCACTAACTTAGACAGGACTAATGTTTCA
AGCCAGACTCCCTCTGCCAAAAGAAGTTTGGGATTTCTTCTCAGCCAG
TTCTCTTTCTGTAAAAAACCTGAGGTGTAACCAGGATTACACTGCCTG
GAATAAACCAAGAGTGCCCTTTCTCTCACCAACAGCAGCAACTGCAG
GGCTTCTCCAATTTGGGAAATACCTGCTATATGAATGCTATTCTACAAT
CTCTATTTTCACTCCAGTCATTTGCAAATGACTTGCTTAAACAAGGTAT
CCCATGGAAGAAAATTCCTACTCAATGCACTTATCAGACGCTTTGCACAC
TTGCTTGTAAAAAAGATATCTGTAATTCAGAGACCAAAAAGGATTTAC
TCAAGAAGGTTAAAATGCCATTTTCAGCTACAGCAGAGAGATTCTCTGG
TTATATGCAGAATGATGCTCATGAATTTTTAAGTCAGTGTGGACCAG
CTGAAAAGAAGATATGGAAAATTAATAAAAACCTTGGAAAGACTGAACCTG
TTTCTGGAGAAGAAAATTCACCAGATATTTTCAGCTACCAGAGCATAAC
TTGCCCTGTTATTACTAATTTGGAGTTTGAGGTTTCAGCACTCCATCATT
TGTAAGCATGTGGAGAGATTATCCCCAAAAGAGAAGCAAGTTAATGACC
TCTCTATTGACCTTCTCCTCGTAGGAAAAAACCACTCCCTCCTCGTTCAAT
TCAAGATTCTCTTGATCTTTTCTTTAGGGCCGAAGAAGTGGAGTATTCT
TGTGAGAAGTGTGGTGGGAAGTGTGCTCTTGTGAGGCACAAATTTAACA
GGCTTCTAGGGTCTCATTCTCCATTTGAAACGATATAGCTTCAATGT
GGCTCTCTCGCTTAAACAATAAGATTGGGCAGCAAGTCATCATTCCAAGA
TACCTGACCCTGTCTCATTGCACTGAAAATACAAAACCACCTTTTA
CCCTTGGTTGGAGTGCACATATGGCAATTTCTAGACCATTGAAAGCCTC
TCAAATGGTGAATTCCTGCATCACCAGCCCTTCTACACCTTCAAAGAAA
TTCACCTTCAAATCCAAGAGCTCCTTGGCTTTATGCCTTGATTTCAGACA
GTGAGGATGAGCTAAAACGTTCTGTGGCCCTCAGCCAGAGACTTTGTGA
AATGTTAGGCAACGAACAGCAGCAGGAAGACCTGGAAAAGATTCAAAA
TTATGCCCAATAGAGCCTGACAAGTCTGAATTGAAAACACTCAGGATTTG
ACAGAATGAGCGAAGAAGAGCTTCTAGCAGCTGTCTTGGAGATAAGTAA
GAGAGATGCTTACCATCTCTGAGTCATGAAGATGATGATAAGCCAAC
AGCAGCCCAGATAACGGATTTGCAGAAGATGATATTCAGAAAATGCCAG
AAAATCCAGACACTATGGAACTGAGAAGCCCAAAAACATCACAGAGCT
GGATCCTGCCAGTTTTACTGAGATAACTAAAGACTGTGATGAGAATAAA
GAAAACAAAACCTCCAGAAGGATCTCAGGGAGAAGTTGATTGGCTCCAGC
AGTATGATATGGAGCGTGAAGGGAAGAGCAAGAGCTTTCAGCAGGCCT
GGCTCAGAGCCTTCAAGAGCAAGAGGCTTGGGAACAGAAAGAAGATGAT
GACCTCAAAGAGCTACCGAGTTAAGTCTTCAAGAGTTTAAACAACCTCCT
TTGTGGATGCATTGGGTTCTGATGAGGACTCTGGAAAATGAGGATGTTTT
TGATATGGAGTACACAGAAGCTGAAGCTGAGGAACTGAAAAGAAATGCT
GAGACAGGAAATCTGCCTCATTCTCAGGTCATTACATTAGTGTATATGA
CATTAAGAAGCAAGCGTGGTTTTACTTACAATGACCTGGAGGTATCAAAA
ATCCAAGAGGCTGCCGTGCAGAGTGATCGAGATCGGAGTGGCTACATCT

Division of Signal Transduction Therapy

TCTTTTATATGCACAAGGAGATCTTTGATGAGCTGCTGGAAACAGAAA
GAACTCTCAGTCACTTAGCACGGAAGTGGGAAGACTACCCGTCAGGCC
TCGtgagcggccgc