Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of USP30 C77A [57 - 517]

Enzyme description:- USP30 C77A [57 – 517]

Clone number:- DU 48346

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 79, 528.00 daltons
Average Mass 79, 579.22 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.83

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

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Clone Data Sheet

USP30 C77A [57 - 517]

Protein	USP30 C77A [57 - 517]
Clone number	DU 48346
Species	Human
Accession number	NM_032663.4
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSAM**TERKKRR** **KGLVPGLVNLGNTAFMNSLLQGLSACPAFIRWLEEFTSQYSRDQKEPP** **SHQYLSLTLLHLLKALSCQEVTDDEVLDASCLLDVLRMYRWQISSFEE** **QDAHELFHVITSSLEDERDRQPRVTHLFDVHSLEQQSEITPKQITCRT** **RGSPHPTSNHWKSQHPFHGRLTSNMVCKHCEHQSPVRFDTFDSLSLSI** **PAATWGHPLTLDHCLHHFISSESVRDVVCDNCTKIEAKGTLNGEKVEH** **QRTTFVKQLKLGKLPQCLCIHLQRLSWSSHGTPLKRHEHVQFNEFLMM** **DIYKYHLLGHKPSQHNPKLNKNPGPTLELQDGPGAPTPVLNQPGAPKT** **QIFMNGACSPSLLPTLSAPMPFPLPVVPDYSSSTYLFRLMAVVVHHGD** **MHSGHFVTYRRSPPSARNPLSTSNQWLWVSDDTVRKASLQEVLSSSAY** **LLFYERVLSRMQHQSQECKSEE**
Native sequence	Amino acids T57 – E517 (end) of human USP30. Residue T234 of the fusion protein is equivalent to T57 of the native enzyme. The GST tag is located at residues 1 – 220. The enzyme has a C77A catalytic inactive mutation. Residue C77 is equivalent to A254 of the fusion protein.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites of pGex 6P1

Nucleotide sequence of insert

ggatccgCCATGACAGAAAGAAAGAAGCGTAGAAAAGGGCTTGTGCCT
GGCCTTGTTAATTTAGGGAACACCGCCTTCATGAACTCCCTGCTACAA
GGCCTGTCTGCCTGTCCTGCTTTCATCAGGTGGCTGGAAGAGTTCACC
TCCCAGTACTCCAGGGATCAGAAGGAGCCCCCCTCACACCAGTATTTA
TCCTTAACACTCTTGCACCTTCTGAAAGCCTTGTCCTGCCAAGAAGTT
ACTGATGATGAGGTCTTAGATGCAAGCTGCTTGTTGGATGTCTTAAGA
ATGTACAGATGGCAGATCTCATCATTTGAAGAACAGGATGCTCACGAA
TTATTCCATGTCATTACCTCGTCATTGGAAGATGAGCGAGACCGCCAG
CCTCGGGTCACACATTTGTTTGATGTGCATTCCCTGGAGCAGCAGTCA
GAAATAACTCCCAAACAAATTACCTGCCGCACAAGAGGGTCACCTCAC
CCCACATCCAATCACTGGAAGTCTCAACATCCTTTTCATGGAAGACTC
ACTAGTAATATGGTCTGCAAACACTGTGAACACCAGAGTCCTGTTCGA
TTTGATACCTTTGATAGCCTTTCACTAAGTATTCCAGCCGCCACATGG
GGTCACCCATTGACCCTGGACCACTGCCTTCACCACTTCATCTCATCA
GAATCAGTGCGGGATGTTGTGTGTGACAACTGTACAAAGATTGAAGCC
AAGGGAACGTTGAACGGGGAAAAGGTGGAACACCAGAGGACCACTTTT
GTTAAACAGTTAAAACTAGGGAAGCTCCCTCAGTGTCTCTGCATCCAC
CTACAGCGGCTGAGCTGGTCCAGCCACGGCACGCCTCTGAAGCGGCAT
GAGCACGTGCAGTTCAATGAGTTCCTGATGATGGACATTTACAAGTAC
CACCTCCTTGGACATAAACCTAGTCAACACAACCCTAAACTGAACAAG
AACCCAGGGCCTACACTGGAGCTGCAGGATGGGCCGGGAGCCCCCACA
CCAGTTCTGAATCAGCCAGGGGCCCCCAAAACACAGATTTTTATGAAT
GGCGCCTGCTCCCCATCTTTATTGCCAACGCTGTCAGCGCCGATGCCC
TTCCCTCTCCCAGTTGTTCCCGACTACAGCTCCTCCACATACCTCTTC
CGGCTGATGGCAGTTGTCGTCCACCATGGAGACATGCACTCTGGACAC
TTTGTCACTTACCGACGGTCCCCACCTTCTGCCAGGAACCCTCTCTCA
ACTAGCAATCAGTGGCTGTGGGTCTCCGATGACACTGTCCGCAAGGCC
AGCCTGCAGGAGGTCCTGTCCTCCAGCGCCTACCTGCTGTTCTACGAG
CGCGTCCTTTCCAGGATGCAGCACCAGAGCCAGGAGTGCAAGTCTGAA
GAAtgagcggccgc