Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of USP30 [57 - 517]

Enzyme description:- USP30 [57 – 517]

Clone number:- DU 48345

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 79, 559.97 daltons
Average Mass 79, 611.28 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 [57 - 517]

Protein	USP30 [57 - 517]
Clone number	DU 48345
Species	Human
Accession number	NM_032663.4
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSAM**TERKKRR KGLVPGLVNLGNTCFMNSLLQGLSACPAFIRWLEEFTSQYSRDQKEPP 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.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites of pGex 6P1

Nucleotide sequence of insert

ggatccGCCATGACAGAAAGAAAGAAGCGTAGAAAAGGGCTTGTGCCT
GGCCTTGTTAATTTAGGGAACACCTGCTTCATGAACTCCCTGCTACAA
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