

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of USP30 [57 - 517]**

<b><u>Enzyme description:-</u></b>	USP30 [57 – 517]
<b><u>Clone number:-</u></b>	DU 36315
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH Sepharose

#### **Calculated molecular mass:-**

Monoisotopic        79, 357.89 daltons  
Average Mass        79, 409.00 daltons  
[cysteines reduced, methionines have not been oxidised]

<b><u>Theoretical pI:-</u></b>	6.83
<b><u>Purity:-</u></b>	>80 %
<b><u>Activation protocol:-</u></b>	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

<b><u>Storage temperature:-</u></b>	-70 °C
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#### **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 36315

**Species** Human

**Accession number** NM\_032663.4

**Tags** N-terminal GST

**Baclovirus  
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE  
GAVLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN  
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY  
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGPLG**STERKKRRKG**  
**LVPGLVNLGNTCFMNSLLQGLSACPAFIRWLEEF**TSQYSRDQKEPPSH  
QYLSLTLHLLKALSCQEVTDDEVLDASCLLDVLRMYRWQISSFEEQD  
AHELFHVI**TSSLEDERDRQPRVTHLFDVHSLEQQSEITPKQITCRTRG**  
SPHPTSNHWKSQHPFHGRLTSNMVCKHCEHQSPVRFDTFDSL SLSIPA  
ATWGHPLTLDHCLHFFISSESVRDVVDNCTKIEAKGTLNGEKVEHQ  
TTFVKQLKLGKLPQCLCIHLQRLSWSSHGTPPKRHEHVQFNEFLMMDI  
YKYHLLGHKPSQHNPKNKPNPGPTLELQDGP GAPT PVLNQP GAPTQI  
FMNGACSP SLLPTLSAPMPFPLPVVPDYSSSTYLFRLMAVVVHGMH  
SGHFVTYRRSPPSARNPLSTSNQWLWVSDDTVRKASLQEVLS SSSAYLL  
FYERVL SRMQHQSQECKSEE

**Native sequence** Amino acids T57 – E517 (end) of human USP30.

Residue T232 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 pFastBac GST 6P1

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**Nucleotide  
sequence of  
insert**

ggatccACAGAAAGAAAGAAGCGTAGAAAAGGGCTTGTGCCTGGCCTT  
GTTAATTTAGGGAACACCTGCTTCATGAACTCCCTGCTACAAGGCCTG  
TCTGCCTGTCCTGCTTTTCATCAGGTGGCTGGAAGAGTTCACCTCCAG  
TACTCCAGGGATCAGAAGGAGCCCCCTCACACCAGTATTTATCCTTA  
ACACTCTTGCACCTTCTGAAAGCCTTGTCTGCAAGAAGTTACTGAT  
GATGAGGTCTTAGATGCAAGCTGCTTGTGGATGTCTTAAGAATGTAC  
AGATGGCAGATCTCATCATTTGAAGAACAGGATGCTCACGAATTATTC  
CATGTCATTACCTCGTCATTGGAAGATGAGCGAGACCGCCAGCCTCGG  
GTCACACATTTGTTTGATGTGCATTCCCTGGAGCAGCAGTCAGAAATA  
ACTCCCAAACAAATTACCTGCCGCACAAGAGGGTCACCTACCCCA  
TCCAATCACTGGAAGTCTCAACATCCTTTTCATGGAAGACTCACTAGT  
AATATGGTCTGCAAACACTGTGAACACCAGAGTCTGTTCGATTTGAT  
ACCTTTGATAGCCTTTCACTAAGTATTCCAGCCGCCACATGGGGTCAC  
CCATTGACCCTGGACCCTGCCTTACCACCTTCATCTCATCAGAATCA  
GTGCGGGATGTTGTGTGTGACAACTGTACAAAGATTGAAGCCAAGGGA  
ACGTTGAACGGGGAAAAGGTGGAACACCAGAGGACCCTTTTGTAAA  
CAGTTAAACTAGGGAAGCTCCCTCAGTGTCTCTGCATCCACCTACAG  
CGGCTGAGCTGGTCCAGCCACGGCACGCCTCTGAAGCGGCATGAGCAC  
GTGCAGTTCAATGAGTTCCTGATGATGGACATTTACAAGTACCACCTC  
CTTGGACATAAACCTAGTCAACACAACCCTAAACTGAACAAGAACCCA  
GGCCTACACTGGAGCTGCAGGATGGGCCGGGAGCCCCACACCAGTT  
CTGAATCAGCCAGGGCCCCAAAACACAGATTTTTTATGAATGGCGCC  
TGCTCCCCATCTTTATTGCCAACGCTGTCAGCGCCGATGCCCTTCCCT  
CTCCAGTTGTTCCCGACTACAGCTCCTCCACATACCTCTTCCGGCTG  
ATGGCAGTTGTCTCCACCATGGAGACATGCACTCTGGACACTTTGTC  
ACTTACCGACGGTCCCACCTTCTGCCAGGAACCCTCTCTCAACTAGC  
AATCAGTGGCTGTGGGTCTCCGATGACACTGTCCGCAAGGCCAGCCTG  
CAGGAGGTCTGTCCTCCAGCGCCTACCTGCTGTTCTACGAGCGCGTC  
CTTCCAGGATGCAGCACCAGAGCCAGGAGTGCAAGTCTGAAGAAtga  
gcgcccgc