

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of STXBP1 [1 – 601]

Enzyme description:- STXBP1 [1 - 601]

Clone number:- DU 38538

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 96, 451.39 daltons

Average Mass 96, 513.19 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.20

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

STXBP1 [1 - 601]

<u>Protein</u>	STXBP1 [1 - 601]
<u>Clone number</u>	DU 38538
<u>Species</u>	Human
<u>Accession number</u>	NM_003165.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWOATFGGGDHPPKSDLEVLV FQGPLGSAGIPGSTRAAAMAP IGLKAVVGEKIMHDVIKKVKKKGEWKVLVVDQLSMRMLSSCCKMTD EGITIVEDINKRREPLPSLEAVYLITPSEKSVHSLISDFKDPPTAKYRA AHVFFTDSCPDALFNELVKSRAAKVIKTLTEINIAFLPYESQVYSLDSA DSFQSFYSPHKAQMKNPILERLAEQIATLCATLKEYPAVRYRGEYKDNA LLAQLIQDKLDAYKADDPTMGEGPDKARSQLLILDRGFDPS SPVLHELT FQAMSYDLLPIENDVYKYETSGIGEARVKEVLLDEDDDLWIALRHKHIA EVSQEVTRSLKDFSSSKRMNTGEKTTMRDLSQMLKKMPQYQKELSKYST HLHLAEDCMKHYQGTVDKLCRVEQDLAMGTDAEGEKIKDPMRAIVPILL DANVSTYDKIRIILLYIFLKNGIT EENLNKLIQHAQIPPEDSEIITNMA HLGVPIVTDSTLRRRSKPERKERISEQTYQLSRWTP I IKDIMEDTIEDK LDTKHYPYISTRSSASFSTTAVSARYGHWKHNKAPGEYRSGPRLIIFIL GGVSLNEMRCAYEVTQANGKWEVLIGSTHILTPTKFLMDLRHPDFRESS RVSFEDQAPTME</p>
<u>Native sequence</u>	<p>Amino acids M1 – E601 (end) of human STXBP1. Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Not1</i> sites of pGEX6P-1

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Nucleotide Sequence of insert

gcggccgcgATGGCCCCATTGGCCTCAAAGCTGTTGTCTGGAGAGAAGATTATGCATGATGTGATA
AAGAAGGTCAAGAAGAAGGGGAATGGAAGGTGCTGGTGGTGGATCAGTTAAGCATGAGGATGCTG
TCCTCCTGCTGCAAGATGACAGACATCATGACCGAGGGCATAACGATTGTGGAAGATATCAATAAG
CGCAGAGAGCCGCTCCCCAGCCTGGAGGCTGTGTATCTCATCACTCCATCCGAGAAGTCCGTCCAC
TCTCTCATCAGTGACTTTAAGGACCCGCCGACTGCTAAATACCGGGCTGCACACGTCTTCTTCACT
GACTCTTGTCCAGATGCCCTGTTTAATGAACTGGTAAAATCCCGAGCAGCCAAAGTCATCAAACT
CTGACGGAAATCAATATTGCATTTCTCCCGTATGAATCCCAGGTCTATTCCCTGGACTCTGCTGAC
TCTTTCCAAAGCTTCTACAGTCCCCACAAGGCTCAGATGAAGAATCCTATACTGGAGCGCCTGGCA
GAGCAGATCGCGACCCTTTGTGCCACCCTGAAGGAGTACCCGGCTGTGCGGTATCGGGGGGAATAC
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ACAATGGGGGAGGGCCCAGACAAGGCACGCTCCCAGCTCCTGATCCTGGATCGAGGCTTTGACCCC
AGCTCCCCTGTGCTCCATGAATTGACTTTTCAGGCTATGAGTTATGATCTGCTGCCTATCGAAAAT
GATGTATACAAGTATGAGACCAGCGGCATCGGGGAGGCACGGGTGAAGGAGGTGCTCCTGGACGAG
GACGACGACCTGTGGATAGCACTGCGCCACAAGCACATCGCAGAGGTGTCCCAGGAAGTCACCCGG
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GAGATCATCACCAACATGGCTCACCTCGGCGTGCCCATCGTCACCGATTCCACGCTGCGTCGCCGG
AGCAAGCCGGAGCGGAAGGAACGCATCAGCGAGCAGACCTACCAGCTCTCACGGTGGACTCCGATT
ATCAAGGACATCATGGAGGACACTATTGAGGACAAACTTGACACCAAACACTACCCTTATATCTCT
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AAGGCCCCAGGCGAGTACCGCAGTGGCCCCCGCCTCATCATTTTCATCCTTGGGGGTGTGAGCCTG
AATGAGATGCGCTGCGCCTACGAGGTGACCCAGGCCAACGGAAAGTGGGAGGTGCTGATAGGTTCT
ACTCACATTCTTACTCCACCAAATTTCTCATGGACCTGAGACACCCCGACTTCAGGGAGTCCTCT
AGGGTATCTTTTGAGGATCAGGCTCCAACAATGGAGtgagcggccgc

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