

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of LST8 [1 - 326]

Enzyme description:- LST8 [1 – 326]

Clone number:- DU 13511

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 63,012.08 daltons

Average Mass 63,052.76 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.67

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

LST8 [1 - 326]

<u>Protein</u>	LST8 [1 - 326]
<u>Clone number</u>	DU 13511
<u>Species</u>	Human
<u>Accession number</u>	NM_022372.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSRIAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLOGWQATFGGGDHPKSDLVPRGSLEVLFGQPMNTSPG TVGSDPVILATAGYDHTVRFWQAHSGICTRTVQHQDSQVNALEVTPDR SMIAAAGYQHIRMIDLNSNNPNPIISYDGVNKNIASVGFHEDGRWMT GGEDCTARIWDLRSRNLQCQRIFQVNAPINCVCLHPNQAEIIVGDQSG AIHIWDLKTDHNEQLIPEPEVSITSAHIDPDASYMAAVNSTGNCYVWN LTGGIGDEVTQLIPKTKIPAHTRYALQCRFSPDSTLLATCSADQTCKI WRTSNFSLMTELSIKSGNPGESSRGWMWGCAFSGDSQYIVTASSDNLA RLWCVETGEIKREYGGHQKAVVCLAFNDSVLG
<u>Native sequence</u>	Amino acids M1 – G326 (end) of human LST8. Residue M235 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	Prescission site (<u>LEVLFQGP</u>) at residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGex4

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Nucleotide

sequence of insert

ggatccctggaagttctgttccagggcccgATGAACACCTCCCCAGGC
ACGGTGGGCAGTGACCCGGTCATCCTGGCCACTGCAGGCTACGACCAC
ACCGTGCGCTTCTGGCAGGCCCACAGCGGCATCTGCACCCGGACGGTG
CAGCACCAGGACTCCCAGGTGAATGCCTTGGAGGTCACACCGGACCGC
AGCATGATTGCTGCTGCAGGTTACCAGCACATCCGCATGTATGATCTC
AACTCCAATAACCCTAACCCCATCATCAGCTACGACGGCGTCAACAAG
AACATCGCGTCTGTGGGCTTCCACGAAGACGGCCGCTGGATGTACACG
GGCGGCGAGGACTGCACAGCCAGGATCTGGGACCTCAGGTCCCGGAAC
CTGCAGTGCCAGCGGATCTTCCAGGTGAACGCACCCATTAAGTGCCTG
TGCCTGCACCCCAACCAGGCAGAGCTCATCGTGGGTGACCAGAGCGGG
GCTATCCACATCTGGGACTTGAAAACAGACCACAACGAGCAGCTGATC
CCTGAGCCCGAGGTCTCCATCACGTCCGCCCACATCGATCCCGACGCC
AGCTACATGGCAGCTGTCAATAGCACCGGAAACTGCTATGTCTGGAAT
CTGACGGGGGGCATTTGGTGACGAGGTGACCCAGCTCATCCCCAAGACT
AAGATCCCTGCCCACACGCGCTACGCCCTGCAGTGTGCTTCAGCCCC
GACTCCACGCTCCTCGCCACCTGCTCGGCTGATCAGACGTGCAAGATC
TGGAGGACGTCCAATTCTCCCTGATGACGGAGCTGAGCATCAAGAGC
GGCAACCCCGGGGAGTCCCTCCCGCGGCTGGATGTGGGGCTGCGCCTTC
TCGGGGGACTCCAGTACATCGTCACTGCTTCCCTCGGACAACCTGGCC
CGGCTCTGGTGTGTGGAGACTGGAGAGATCAAGAGAGAGTATGGCGGC
CACCAGAAGGCTGTTGTCTGCCTGGCCTTCAATGACAGTGTGCTGGGC
tagcggccgc