

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of LEF1 [1 – 399]

<u>Enzyme description:-</u>	LEF1 [1 - 399]
<u>Clone number:-</u>	DU 32823
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 70, 979.38 daltons
Average Mass 71, 024.80 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.48

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

LEF1 [1 - 399]

<u>Protein</u>	LEF1 [1 - 399]
<u>Clone number</u>	DU 32823
<u>Species</u>	Human
<u>Accession number</u>	NM_016269.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWOATFGGGDHPPKSDLEVLFGQPLGSMPQLSGGGGGGGGD PELCATDEMIPFKDEGDPQKEKIFAEISHPEEEGDLADIKSSLVNESEI IPASNGHEVARQAQTSQEPYHDKAREHPDDGKHDPDGLYNKGPSYSSYS GYIMPMNMNDPYMSNGSLSPPIPRTSNKVPVVQPSHAVHPLTPLITYS DEHFSPGSHPSHIPSDVNSKQGM SRHPPAPDIPTFYPLSPGGVGQITPP LGWQGQPVYPITGGFRQPYPSSLSVDTSMSRFSHHMIPGPPGPHTTGIP HPAIVTPQVKQEHPTDSDLMHVKPQHEQRKEQEPKRPHIKKPLNAFML YMKEMRANVVAECTLKESAAINQILGRRWHALSREEQAKYYELARKERQ LHMQLYPGWSARDNYGKKKKRKREKLQESASGTGPRMTAAYI
<u>Native sequence</u>	Amino acids M1 – I399 (end) of human LEF1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (LEVLFGQP) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P-1

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Nucleotide

Sequence of insert

ggatccATGCCCCAACTCTCCGGAGGAGGTGGCGGCGGCGGGGGGGACC
CGGAACTCTGCGCCACGGACGAGATGATCCCCCTTCAAGGACGAGGGCGA
TCCTCAGAAGGAAAAGATCTTCGCCGAGATCAGTCATCCCGAAGAGGAA
GGCGATTTAGCTGACATCAAGTCTTCCTTGGTGAACGAGTCTGAAATCA
TCCCGGCCAGCAACGGACACGAGGTGGCCAGACAAGCACAAACCTCTCA
GGAGCCCTACCACGACAAGGCCAGAGAACACCCCGATGACGGAAAGCAT
CCAGATGGAGGCCTCTACAACAAGGGACCCTCCTACTCGAGTTATTCCG
GGTACATAATGATGCCAAATATGAATAACGACCCATACATGTCAAATGG
ATCTCTTTCTCCACCCATCCCGAGAACATCAAATAAAGTGCCCGTGGTG
CAGCCATCCCATGCGGTCCATCCTCTCACCCCCCTCATCACTTACAGTG
ACGAGCACTTTTCTCCAGGATCACACCCGTACACATCCCATCAGATGT
CAACTCCAAACAAGGCATGTCCAGACATCCTCCAGCTCCTGATATCCCT
ACTTTTTATCCCTTGTCTCCGGGTGGTGTGGACAGATCACCCACCTC
TTGGCTGGCAAGGTCAGCCTGTATATCCCATCACGGGTGGATTCAGGCA
ACCCTACCCATCCTCACTGTCTAGTCGACACTTCCATGTCCAGGTTTTCC
CATCATATGATTCCCGGTCCTCCTGGTCCCCACACAACTGGCATCCCTC
ATCCAGCTATTGTAACACCTCAGGTCAAACAGGAACATCCCCACACTGA
CAGTGACCTAATGCACGTGAAGCCTCAGCATGAACAGAGAAAGGAGCAG
GAGCCAAAAGACCTCACATTAAGAAGCCTCTGAATGCTTTTATGTTAT
ACATGAAAGAAATGAGAGCGAATGTCGTTGCTGAGTGTACTCTAAAAGA
AAGTGCAGCTATCAACCAGATTCTTGGCAGAAGGTGGCATGCCCTCTCC
CGTGAAGAGCAGGCTAAATATTATGAATTAGCACGGAAAGAAAGACAGC
TACATATGCAGCTTTATCCAGGCTGGTCTGCAAGAGACAATTATGGTAA
GAAAAGAAGAGGAAGAGAGAGAACTACAGGAATCTGCATCAGGTACA
GGTCCAAGAATGACAGCTGCCTACATCtgagcgccgc