Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of RILPL1 [1 - 403]

Enzyme description:- RILPL1 [1 - 403]

Clone number:- DU 26577

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His6-SUMO

Purification method:- Ni^{2+}-NTA Agarose

Calculated molecular mass:-
Monoisotopic 59, 065.86 daltons
Average Mass 59, 102.25 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.30

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

Division of Signal Tranduction Therapy

Clone Data Sheet

RILPL1 [1 - 403]

Protein	RILPL1 [1 - 403]
Clone number	DU 26577
Species	Human
Accession number	NM_178314.4
Tags	N-terminal His-SUMO
Bacterially expressed protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVY QEQTGG**MEEERGSALAAESALEKNVAELTVMDVYDIASLVGHEFERVID** **QHGCEAIARLMPKVVRVLEILEVLVSRHHVAPELDELRLELDRLRLERM** **DRIEKERKHQKELELVEDVWRGEAQDLLSQIAQLQEENKQLMTNLSHKD** **VNFSEEEFQKHEGMSERERQVMKKLKEVVDKQRDEIRAKDRELGLKNED** **VEALQQQQTRLMKINHDLRHRVTVVEAQGKALIEQKVELEADLQTKEQE** **MGSLRAELGKLRERLQGEHSQNGEEEPETEPVGEESISDAEKVAMDLKD** **PNRPRFTLQELRDVLHERNELKSKVFLLQEELAYYKSEEMEEENRIPQP** **PPIAHPRTSPQPESGIKRLFSFFSRDKKRLANTQRNVHIQESFGQWANT** **HRDDGYTEQGQEALQHL**
Native sequence	Amino acids M1 – L403 (end) of human RILPL1. Residue M105 of the fusion protein is equivalent to M1 of the native enzyme. The His6 tag is located at residues 3 – 8.
Protease cleavage	SENP1 cleavage of SUMO: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG) residues 9 - 104
Cloning sites	*Eco*R1 and *Not*1 sites of pET15b His6-SUMO

Nucleotide Sequence Of Insert

gaattcATGGAGGAGGAGCGGGGGTCGGCGCTGGCGGCCGAGTCGGCGCTGGAGAAGAA
CGTGGCCGAGCTGACCGTCATGGACGTGTACGACATCGCGTCGCTTGTGGGCCACGAGT
TCGAGCGGGTCATTGACCAGCACGGCTGCGAGGCCATCGCGCGCCTCATGCCCAAGGTC
GTGCGCGTCCTGGAGATCCTGGAGGTGCTGGTCAGCCGCCACCACGTCGCGCCCGAGCT
GGACGAGCTGCGCCTGGAGCTGGACCGCCTGCGCCTGGAGAGGATGGACCGCATCGAGA
AGGAGCGCAAGCACCAGAAGGAGCTGGAGCTGGTGGAGGATGTGTGGCGAGGGGAGGCG
CAGGACCTCCTCTCCCAGATCGCCCAGCTGCAGGAGGAGAACAAGCAGCTCATGACCAA
CCTCTCCCACAAGGATGTCAACTTCTCAGAGGAGGAGTTCCAGAAGCATGAAGGCATGT
CAGAGCGGGAGCGACAGGTGATGAAGAAGCTGAAGGAGGTGGTGGACAAACAACGCGAC
GAGATCCGCGCCAAGGACAGGGAGCTGGGCCTGAAAAATGAGGACGTTGAGGCTTTACA
GCAGCAGCAGACACGGCTGATGAAGATCAACCATGACCTTCGGCACCGGGTCACGGTGG
TGGAGGCCCAGGGGAAAGCCCTGATCGAACAGAAGGTGGAGCTGGAGGCAGACCTGCAG
ACCAAGGAGCAGGAGATGGGCAGCCTGCGAGCAGAGCTGGGGAAGTTGCGAGAGAGGCT
GCAGGGGGAGCACAGCCAGAATGGGGAGGAGGAGCCTGAGACGGAGCCGGTGGGAGAGG
AGAGCATCTCCGACGCAGAGAAGGTGGCCATGGATCTCAAGGACCCCAACCGCCCCCGG
TTCACCCTGCAGGAGCTGCGGGACGTGCTGCACGAGAGGAACGAGCTCAAGTCCAAGGT
GTTCTTGCTGCAGGAGGAGCTGGCTTACTATAAGAGTGAAGAAATGGAAGAGGAAAACC
GAATACCCCAACCCCCACCCATCGCCCACCCGAGGACGTCCCCCCAGCCGGAGTCGGGC
ATCAAGCGACTGTTTAGCTTCTTCTCCCGAGATAAGAAGCGCCTGGCCAACACACAGAG
AAACGTGCACATCCAGGAGTCCTTTGGACAGTGGGCAAACACCCACCGCGATGACGGTT
ACACAGAGCAAGGACAGGAAGCCCTGCAGCATCTGtgagcggccgcgtaaagatccggc
tgctaacaaagcccgaaagagctgggggtgggctcct