

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RILPL2 [1 - 120]

<u>Enzyme description:-</u>	RILPL2 [1 - 120]
<u>Clone number:-</u>	DU 27504
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal His6-SUMO
<u>Purification method:-</u>	Ni ²⁺ -NTA Agarose

Calculated molecular mass:-

Monoisotopic 25, 512.59 daltons

Average Mass 25, 528.50 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.84

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

RILPL2 [1 - 120]

Protein RILPL2 [1 - 120]

Clone number DU 27504

Species Human

Accession number NM_145058.2

Tags N-terminal His-SUMO

Bacterially expressed protein
MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTT
HLKCLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEDVIEVY
QEQTGGMEPPVREEEEEEGEEDERDEVGPEGALGKSPFQLTAEDVYD
**ISYLLGRELMALGSDPRVTQLQFKVVRVLEMLEALVNEGSLALEELKME
RDHLRKEVEGLRRQSPASGEVNLGPNK**

Native sequence Amino acids M1 – K120 (end residue T211) of human RILPL2.
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:
(SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTT
HLKCLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME
EEDVIEVYQEQTGG) residues 9 - 104

Cloning sites *Bam*H1 and *Not*1 sites of pET15b His6-SUMO

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Nucleotide
Sequence Of
Insert

ggatccATGGAGGAGCCCCCTGTGCGAGAAGAGGAAGAGGAGGAGGGAG
AGGAGGACGAGGAGAGGGACGAGGTTGGGCCCGAGGGGGCGCTGGGCAA
GAGCCCCCTCCAGCTGACCGCCGAGGACGTGTATGACATCTCCTACCTG
TTGGGCCGCGAGCTTATGGCCCTGGGCAGCGACCCCCGGGTGACGCAGC
TGCAGTTCAAAGTCGTCCGCGTCCTGGAGATGCTGGAGGCGCTGGTGAA
TGAGGGCAGCCTGGCGCTGGAGGAGCTGAAGATGGAGAGGGACCACCTC
AGGAAGGAGGTGGAGGGGCTGCGGAGACAGAGCCCTCCGGCCAGCGGGG
AGGTGAACCTGGGCCCAAACAAAtagcggccgc