*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

# Preparation of RILPL1 [1 - 190]

**Enzyme description:-** RILPL1 [1 - 190]

**Clone number:-** DU 26803

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal His6-SUMO

**Purification method:-** $\text{Ni}^{2+}$-NTA Agarose

**Calculated molecular mass:-**
Monoisotopic 34, 303.33 daltons
Average Mass 34, 324.76 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

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# Clone Data Sheet

# RILPL1 [1 - 190]

**Protein** RILPL1 [1 - 190]

**Clone number** DU 26803

**Species** Human

**Accession number** NM_178314.4

**Tags** N-terminal His-SUMO

**Bacterially expressed protein**

MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT
HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVY
QEQTGG**MEEERGSALAAESALEKNVAELTVMDVYDIASLVGHEFERVID**
**QHGCEAIARLMPKVVRVLEILEVLVSRHHVAPELDELRLELDRLRLERM**
**DRIEKERKHQKELELVEDVWRGEAQDLLSQIAQLQEENKQLMTNLSHKD**
**VNFSEEEFQKHEGMSERERQVMKKLKEVVDKQRDEIRAKDRELGLKNED**

**Native sequence** Amino acids M1 – D190 (end residue L403) 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**

```
gaattcATGGAGGAGGAGCGGGGGTCGGCGCTGGCGGCCGAGTCGGCGC
TGGAGAAGAACGTGGCCGAGCTGACCGTCATGGACGTGTACGACATCGC
GTCGCTTGTGGGCCACGAGTTCGAGCGGGTCATTGACCAGCACGGCTGC
GAGGCCATCGCGCGCCTCATGCCCAAGGTCGTGCGCGTCCTGGAGATCC
TGGAGGTGCTGGTCAGCCGCCACCACGTCGCGCCCGAGCTGGACGAGCT
GCGCCTGGAGCTGGACCGCCTGCGCCTGGAGAGGATGGACCGCATCGAG
AAGGAGCGCAAGCACCAGAAGGAGCTGGAGCTGGTGGAGGATGTGTGGC
GAGGGGAGGCGCAGGACCTCCTCTCCCAGATCGCCCAGCTGCAGGAGGA
GAACAAGCAGCTCATGACCAACCTCTCCCACAAGGATGTCAACTTCTCA
GAGGAGGAGTTCCAGAAGCATGAAGGCATGTCAGAGCGGGAGCGACAGG
TGATGAAGAAGCTGAAGGAGGTGGTGGACAAACAACGCGACGAGATCCG
CGCCAAGGACAGGGAGCTGGGCCTGAAAAATGAGGACtgagaggct
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