Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of RILPL2 [1 - 211]

Enzyme description:-	RILPL2 [1 - 211]
Clone number:-	DU 27483
Source:-	Recombinant
Expression system:-	*E.coli*
Tag:-	N-terminal His6-SUMO
Purification method:-	Ni^{2+}-NTA Agarose

Calculated molecular mass:-

Monoisotopic	35, 958.25 daltons
Average Mass	35, 980.59 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	5.29
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 - 211]

Protein	RILPL2 [1 - 211]
Clone number	DU 27483
Species	Human
Accession number	NM_145058.2
Tags	N-terminal His-SUMO
Bacterially expressed protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVY QEQTGG**MEEPPVREEEEEEGEEDEERDEVGPEGALGKSPFQLTAEDVYD** **ISYLLGRELMALGSDPRVTQLQFKVVRVLEMLEALVNEGSLALEELKME** **RDHLRKEVEGLRRQSPPASGEVNLGPNKMVVDLTDPNRPRFTLQELRDV** **LQERNKLKSQLLVVQEELQCYKSGLIPPREGPGGRREKDAVVTSAKNAG** **RNKEEKTIIKKLFFFRSGKQT**
Native sequence	Amino acids M1 – T211 (end) 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: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG) residues 9 - 104
Cloning sites	*Bam*H1 and *Not*1 sites of pET15b His6-SUMO

Nucleotide Sequence Of Insert

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ggatccATGGAGGAGCCCCCTGTGCGAGAAGAGGAAGAGGAGGAGGGAG
AGGAGGACGAGGAGAGGGACGAGGTTGGGCCCGAGGGGGCGCTGGGCAA
GAGCCCCTTCCAGCTGACCGCCGAGGACGTGTATGACATCTCCTACCTG
TTGGGCCGCGAGCTTATGGCCCTGGGCAGCGACCCCCGGGTGACGCAGC
TGCAGTTCAAAGTCGTCCGCGTCCTGGAGATGCTGGAGGCGCTGGTGAA
TGAGGGCAGCCTGGCGCTGGAGGAGCTGAAGATGGAGAGGGACCACCTC
AGGAAGGAGGTGGAGGGGCTGCGGAGACAGAGCCCTCCGGCCAGCGGGG
AGGTGAACCTGGGCCCAAACAAAATGGTGGTTGACCTGACAGATCCCAA
CCGACCCCGCTTCACTCTGCAGGAGCTAAGGGATGTGCTGCAGGAACGC
AACAAACTCAAGTCGCAGCTCCTGGTGGTGCAGGAAGAGCTGCAGTGCT
ACAAGAGTGGCCTGATTCCACCAAGAGAAGGCCCAGGAGGAAGAAGAGA
AAAAGATGCTGTGGTTACTAGTGCCAAAAATGCTGGCAGGAACAAGGAG
GAGAAGACAATCATAAAAAAGCTGTTCTTTTTTCGATCGGGGAAACAGA
CCtaggcggccgc
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