

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB3IP [1 – 476]

<u>Enzyme description:-</u>	RAB3IP [1 – 476]
<u>Clone number:-</u>	DU 21167
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i> ,
<u>Tag:-</u>	N-terminal His(6) - SUMO
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose, Cleavage of His6-SUMO and Gel filtration
<u>Calculated molecular mass:-</u>	
Monoisotopic	52, 987.71 daltons [After tag cleavage]
Average Mass	53, 021.21 daltons [After tag cleavage]
	[cysteines reduced, methionines have not been oxidised]
<u>Theoretical pI:-</u>	5.83
<u>Purity:-</u>	>80 %
<u>Activation Protocol:-</u>	Expressed in the presence of GroEL / GroES
<u>Enzyme storage buffer:-</u>	50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF
<u>Storage temperature:-</u>	-70 °C

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Clone Data Sheet

RAB3IP [1 - 476]

Protein RAB3IP [1 - 476]

Clone number DU 21167

Species Human

Accession number NM_175623.3

Tags N-terminal His(6) + SUMO

**Bacterially
expressed RAB3IP
protein**

MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMT
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE
VYQEQTGGMGLKKMKGLSYDEAFAMANDPLEGFHEVNLASPTSPDLLG
VYESGTQEQTSPSVIYRPHPSALSSVPIQANALDVSELPTQPVSPP
RRLNCAEISSISFHVTDPAFCSTSGVTAGLTKLTTRKDNNAEREFLO
GATITEACDGSDDIFGLSTDSLRLRSPSVLEVREKGYERLKEELAKA
QRELKLDDEECERLSKVRDQLGQELEELTASLFEEAHKVVREANIKQA
TAEKQLKEAQGKIDVLAQAEVAALKTLVLSSTPTQEPPLGGKTPFK
KGHTRNKSTSSAMSGSHQDLSVIQPIVKDCKEADLSLYNEFRLWKDEP
TMDRTPFLDKIYQEDIFPCLTFKSELASAVLEAVENNTLSIEPVGL
QPIRFVKASAVECGGPKKCALTGQSKSKHRIKLGSSNYYYISPFGR
YRITSVCNFFTYIRYIQOGLVKQDQVDFWEVMDLRKEMSLAKLGYF
KEEL

Native sequence Amino acids M1 – L476 (end) of human RAB3IP.
Residue M105 of the fusion protein is equivalent to M1 of the native
enzyme. The His(6) tag is located at residues 2 – 7.

Protease cleavage SENP1 cleavage of SUMO:
(SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTT
HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME
EEDVIEVYQEQTGG) residues 9 - 104

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

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**Nucleotide
sequence of insert**

ggatccATGGGATTAAAAAAGATGAAAGGGTTATCTTATGATGAGGCT
TTTGCTATGGCTAATGATCCCTTGGAAGGCTTCCATGAAGTAAACCTT
GCTTCACCTACTTCTCCGGACCTTCTTGGTGTGTATGAATCAGGAACT
CAAGAGCAGACTACCTCACCAAGTGTATCTACCGGCCACACCCTTCA
GCTTTATCCTCTGTACCTATCCAGGCAAATGCATTAGATGTTTCTGAA
CTTCCTACACAACCCGTGTATTCATCCCCAGACGTTTAAATTGTGCG
GAAATATCTAGTATCAGCTTTCATGTTACAGACCCAGCCCCTTGCTCT
ACCTCTGGAGTCACAGCTGGATTAACTAAATTAACTACAAGAAAGGAC
AACTATAATGCAGAGAGAGAGTTTTTACAGGGTGCTACTATAACAGAG
GCTTGCGATGGCAGTGATGATATTTTTGGGTTGAGTACTGATAGTCTG
TCTCGTTTACGAAGCCCATCTGTTTTGGAAGTTAGAGAAAAGGGCTAT
GAACGATTAAGAAGAACTCGCAAAGCTCAGAGGGAACCTGAAGTTA
AAAGATGAAGAATGTGAGAGGCTTTCAAAAGTGCGAGATCAACTTGGA
CAGGAATTGGAAGAACTCACAGCTAGTCTATTTGAGGAAGCTCATAAA
ATGGTGAGAGAAGCAAATATCAAGCAGGCAACAGCAGAAAAACAGCTA
AAAGAAGCACAAGGAAAAATTGATGTACTTCAAGCTGAAGTAGCTGCA
TTGAAGACACTTGTATTGTCCAGTTCTCCAACATCACCTACGCAGGAG
CCTTTGCCAGGTGGAAGACACCTTTTTAAAAGGGGCATACAAGAAAT
AAAAGCACAAGCAGTGCTATGAGTGGCAGTCATCAGGACCTCAGTGTG
ATACAGCCAATTGTAAAAGACTGCAAAGAGGCTGACTTATCCTTGTAT
AATGAATTCCGATTGTGGAAGGATGAGCCACAATGGACAGGACGTGT
CCTTTCTTAGACAAAATCTACCAGGAAGATATCTTTCCATGTTTAAACA
TTCTCAAAAAGTGAGTTGGCTTCAGCTGTTCTGGAGGCTGTGAAAAC
AATACTCTAAGCATTGAACCAGTGGGATTACAACCTATCCGGTTTGTG
AAAGCTTCTGCAGTTGAATGCGGAGGACCAAAAAAATGTGCTCTCACT
GGCCAGAGTAAGTCCTGTAAACACAGAATTAAATTAGGGGACTCAAGC
AACTATTATTATATTTCTCCTTTTTTGCAGATACAGGATCACTTCTGTA
TGTAACCTTTTTTACATACATTCGATACATTCAGCAGGGACTCGTGAAA
CAGCAGGATGTTGATCAGATGTTTTGGGAGGTTATGCAGTTGAGAAAA
GAGATGTCATTGGCAAAGCTGGGTTATTTCAAAGAGGAACTCtgagcg
gccgc