

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of RAB19 [1 – 217]**

<b><u>Enzyme description:-</u></b>	RAB19 [1 – 217]
<b><u>Clone number:-</u></b>	DU 26452
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	<i>E.coli</i> ,
<b><u>Tag:-</u></b>	N-terminal His(6) - SUMO
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose, Cleavage of His6-SUMO and Gel filtration
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	24, 384.29 daltons [After tag cleavage]
Average Mass	24, 399.85 daltons [After tag cleavage]
	[cysteines reduced, methionines have not been oxidised]
<b><u>Theoretical pI:-</u></b>	6.06 [After tag cleavage]
<b><u>Purity:-</u></b>	>80 %
<b><u>Activation Protocol:-</u></b>	Expressed in the presence of GroEL / GroES
<b><u>Enzyme storage buffer:-</u></b>	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
<b><u>Storage temperature:-</u></b>	-70 °C

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**Clone Data Sheet**

**RAB19 [1 - 217]**

**Protein** RAB19 [1 - 217]

**Clone number** DU 26452

**Species** Human

**Accession number** A4D1S5

**Tags** N-terminal His(6) + SUMO

**Bacterially expressed RAB19 protein**  
MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMT  
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE  
VYQEQTGGMHFSSSARAADENFDYLFKIILIGDSNVGKTCVVQHFKSG  
VYTETQONTIGVDFTVRSLDIDGKKVKMQVWDTAGQERFRTITQSYR  
SAHAAI IAYDLTRRSTFESIPHWIHEIEKYGAANVIMLIGNKDLWE  
KRHVLFEDACTLAEKYGLLAVLETSAKESKNIEEVFVLMAKELIARNS  
LHLYGESALNGLPLDSSPVLMAQGPSEKTHCTC

**Native sequence** Amino acids M1 – C217 (end) of human RAB19.  
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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**Complete  
Nucleotide  
Sequence**

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA  
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAA  
GTCATTGGACAGGATAGCAGTGAGATTCACCTCAAAGTGAAAATGACA  
ACACATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT  
CCAATGAACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT  
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA  
GTTTATCAGGAACAAACGGGGGAATGCACTTCTCCAGCTCAGCCAGG  
GCAGCAGATGAGAACTTTGACTATTTGTTCAAGATTATCCTCATTTGG  
GATTCCAATGTGGGAAGACGTGTGTGGTGCAGCATTTCAAGTCTGGA  
GTCTACACTGAGACACAGCAGAACACGATTGGAGTGGACTTTACCGTG  
CGTTCCCTTGATATTGACGGCAAAAAAGTGAAGATGCAGGTGTGGGAC  
ACAGCTGGCCAGGAGCGCTTCCGCACCATACCCAAAGCTACTACCGC  
AGTGCCACGCAGCCATCATCGCCTATGACCTCACCCGGCGGTCCACG  
TTCGAGTCCATCCCTCACTGGATTCATGAGATAGAGAAATATGGAGCT  
GCAAATGTGGTCATTATGCTGATTGGAAATAAATGTGACCTCTGGGAA  
AAGCGGCACGTCTTGTTCGAGGATGCCTGCACACTGGCTGAGAAGTAC  
GGCTCCTGGCCGTTTTTGAGACATCTGCCAAGGAGTCAAAGAACATA  
GAAGAAGTCTTCGTGCTCATGGCCAAGGAGCTGATCGCGCGCAACAGC  
CTGCACCTATATGGGGAGAGTGCCCTGAACGGCTCCCCCTGGACTCC  
AGCCCCGTTCTTATGGCCAGGGTCCAAGTGAAAAGACCCACTGCACT  
TGCTaagcggccgc