

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of RAB3D [1 – 219]**

<b><u>Enzyme description:-</u></b>	RAB3D [1 – 219]
<b><u>Clone number:-</u></b>	DU 26454
<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, 251.81 daltons [After tag cleavage]
Average Mass	24, 267.14 daltons [After tag cleavage]
	[cysteines reduced, methionines have not been oxidised]
<b><u>Theoretical pI:-</u></b>	4.76 [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

*Division of Signal Transduction Therapy*

**Clone Data Sheet**

**RAB3D [1 - 219]**

**Protein** RAB3D [1 - 219]

**Clone number** DU 26454

**Species** Human

**Accession number** NM\_004283.3

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

**Bacterially expressed RAB3D protein**  
MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMT  
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE  
VYQEQTGG**MASAGDTQAGPRDAADQNFDMFKLLIGNSSVGKTSFLF**  
**RYADDSFTPAFVSTVGIDFKVKTVYRHDKRIKLQIWDTAGQERYRTIT**  
**TAYYRGAMGFLLMYDIANQESFAAVQDWATQIKTYSWDNAQVILVGNK**  
**CDLEDERVVP AEDGRR LADDLGFEFF EASAKENINVKQVFERLVDVIC**  
**EKMNESLEPSSSSSGSNGKGPVAVGDAPAPQPSSCSC**

**Native sequence** Amino acids M1 – C219 (end) of human RAB3D.  
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*I sites of pET15b His6-SUMO

*Division of Signal Transduction Therapy*

Complete  
Nucleotide  
Sequence

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA  
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAACCTCAA  
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA  
ACACATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT  
CCAATGAACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT  
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA  
GTTTATCAGGAACAAACGGGGGAATGGCATCAGCTGGAGACACCCAG  
GCAGGCCACGGGATGCAGCAGATCAGAACTTCGACTATATGTTCAA  
CTGCTACTGATAGGCAACAGCAGTGTGGGCAAGACTTCCTTCCCTGTT  
CGATACGCGGACGACTCCTTCACTCCCGCCTTCGTCAGTACTGTGGGC  
ATCGATTTCAAGGTCAAGACCGTCTACCGCCATGACAAGAGGATCAAG  
CTGCAGATCTGGGACACAGCGGGCCAGGAGCGCTACCGCACCATCACC  
ACGGCCTACTACCGGGGAGCCATGGGCTTCCTGCTCATGTATGACATC  
GCCAATCAGGAATCCTTTGCCGCTGTGCAGGACTGGGCCACGCAAATC  
AAGACCTACTCCTGGGACAACGCCCAGGTCATCCTGGTGGGGAACAAG  
TGTGACCTGGAGGACGAACGTGTTGTGCCTGCTGAGGATGGCCGGAGG  
CTCGCCGACGACCTTGGTTTCGAGTTCTTTGAAGCCAGTGCCAAGGAG  
AACATCAATGTGAAGCAGGTCTTCGAGCGCCTGGTGGATGTCATCTGC  
GAGAAGATGAACGAGTCCCTGGAACCCAGCTCCAGCTCAGGCAGCAAC  
GGGAAAGGCCCGGCCGTGGGGGATGCTCCAGCCCCCAGCCAGCAGC  
TGCAGCTGctaggcggccgc