

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of RAB7A [1 – 207]**

<b><u>Enzyme description:-</u></b>	RAB7A [1 – 207]
<b><u>Clone number:-</u></b>	DU 24781
<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	23, 474.84 daltons [After tag cleavage]
Average Mass	23, 489.75 daltons [After tag cleavage]
	[cysteines reduced, methionines have not been oxidised]
<b><u>Theoretical pI:-</u></b>	6.39 [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**

**RAB7A [1 - 207]**

<b><u>Protein</u></b>	RAB7A [1 - 207]
<b><u>Clone number</u></b>	DU 24781
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_004637.5
<b><u>Tags</u></b>	N-terminal His(6) + SUMO
<b><u>Bacterially expressed RAB7A protein</u></b>	<b>MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGG<b>MTSRKKVLLKVI ILGDSGVGKTSLMNQYVNKKFSNQYKAT IGADFLTKEVMVDDRLVTMQIWDTAGQERFQSLGVAFYRGADCCVLVF DVTAPNTFKTLDSWRDEFLIQASPRDPENFPFVVLGNKIDLENRQVAT KRAQAWCYSKNNIPYFETSAKEAINVEQAFQTIARNALKQETEVLYN EFPEPIKLDKNDRAKASAESCSC</b></b>
<b><u>Native sequence</u></b>	Amino acids M1 – C207 (end) of human RAB7A. Residue M105 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 – 7.
<b><u>Protease cleavage</u></b>	SEN1 cleavage of SUMO: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG) residues 9 - 104
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pET15b His6-SUMO

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**Complete  
Nucleotide  
Sequence**

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA  
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAA  
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA  
ACACATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT  
CCAATGAACTCACTCAGGTTTTCTCTTTGAGGGTCAGAGAATTGCTGAT  
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA  
GTTTATCAGGAACAAACGGGGGAATGACCTCTAGGAAGAAAAGTGTTG  
CTGAAGGTTATCATCCTGGGAGATTCTGGAGTCGGGAAGACATCACTC  
ATGAACCAGTATGTGAATAAGAAATTCAGCAATCAGTACAAAGCCACA  
ATAGGAGCTGACTTTCTGACCAAGGAGGTGATGGTGGATGACAGGCTA  
GTCACAATGCAGATATGGGACACAGCAGGACAGGAACGGTTCCAGTCT  
CTCGGTGTGGCCTTCTACAGAGGTGCAGACTGCTGCGTTCTGGTATTT  
GATGTGACTGCCCCAACACATTCAAAACCCTAGATAGCTGGAGAGAT  
GAGTTTCTCATCCAGGCCAGTCCCCGAGATCCTGAAAAC TTCCCATTT  
GTTGTGTTGGGAAACAAGATTGACCTCGAAAACAGACAAGTGGCCACA  
AAGCGGGCACAGGCCTGGTGCTACAGCAAAAACAACATTCCCTACTTT  
GAGACCAGTGCCAAGGAGGCCATCAACGTGGAGCAGGCGTTCCAGACG  
ATTGCACGGAATGCACTTAAGCAGGAAACGGAGGTGGAGCTGTACAAC  
GAATTTCTGAACCTATCAAACCTGGACAAGAATGACCGGGCCAAGGCC  
TCGGCAGAAAGCTGCAGTTGCtgagcggccgc