

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB8B [1 – 207]

<u>Enzyme description:-</u>	RAB8B [1 – 207]
<u>Clone number:-</u>	DU 60928
<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	23, 569.08 daltons [After tag cleavage]
Average Mass	23, 584.10 daltons [After tag cleavage]
	[cysteines reduced, methionines have not been oxidised]
<u>Theoretical pI:-</u>	9.15 [After tag cleavage]
<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

RAB8B [1 - 207]

<u>Protein</u>	RAB8B [1 - 207]
<u>Clone number</u>	DU 60928
<u>Species</u>	Human
<u>Accession number</u>	NM_016530.2
<u>Tags</u>	N-terminal His(6) + SUMO
<u>Bacterially expressed RAB8B protein</u>	MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVQMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGGMAKTYDYLFKLLIGDSGVGKTCLLFRFSEDAFNTTFI TIGIDFKIRTIELDGKKIKLQIWDTAGQERFRTITTAYRGAMGIMLY DITNEKSFDNIKNWIRNIEHASSDVERMILGNKCDMNDKRQVSKERG EKLAIDYGIKFLETSAKSSANVEEAFFTLARDIMTKLNRKMNDSNSAG AGGPVKITENRSKKTSFFRCSLL
<u>Native sequence</u>	Amino acids M1 – L207 (end) of human RAB8B. Residue M105 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 – 7.
<u>Protease cleavage</u>	SEN1 cleavage of SUMO: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG) residues 9 - 104
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pET15b His6-SUMO

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**Complete
Nucleotide
Sequence**

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAA
GTCATTGGACAGGATAGCAGTGAGATTCACCTCAAAGTGCAAATGACA
ACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAATTCACCTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGGATCCATGGCGAAGACGTACGATTAT
CTCTTCAAGCTCCTGCTGATCGGCGACTCGGGGTAGGCAAGACCTGC
CTCCTGTTCCGCTTCTCAGAGGACGCCTTCAACACCACCTTCATCTCC
ACCATCGGAATTGATTTTAAATTAGAACGATAGAACTAGATGGAAAG
AAAATTAAGCTTCAGATATGGGACACAGCGGGTCAGGAAAGATTCCGA
ACAATCACGACAGCGTACTACAGAGGAGCCATGGGCATTATGCTGGTC
TATGACATCACAAATGAAAAATCCTTTGACAATATTAATAAATTGGATC
AGAAACATTGAAGAGCATGCCTCTTCCGATGTCGAAAGAATGATCCTG
GGTAACAAATGTGATATGAATGACAAAAGACAAGTGTCAAAAGAAAGA
GGGAGAAAGCTAGCAATTGACTATGGGATTAAATTCCTGGAGACAAGC
GCAAATCCAGTGCAAATGTAGAAGAGGCATTTTTTACACTTGCACGA
GATATAATGACAAAACCTAACAGAAAAATGAATGACAGCAATTCAGCA
GGAGCAGGTGGACCAGTGAAAATAACAGAAAACCGATCAAAGAAGACC
AGTTTCTTTCGTTGCTCGCTACTTtga