

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB1A [1 – 205]

<u>Enzyme description:-</u>	RAB1A [1 – 205]
<u>Clone number:-</u>	DU 26449
<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	22, 663.38 daltons [After tag cleavage]
Average Mass	22, 677.75 daltons [After tag cleavage]
	[cysteines reduced, methionines have not been oxidised]
<u>Theoretical pI:-</u>	5.93 [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

RAB1A [1 - 205]

<u>Protein</u>	RAB1A [1 - 205]
<u>Clone number</u>	DU 26449
<u>Species</u>	Human
<u>Accession number</u>	P62820.3
<u>Tags</u>	N-terminal His(6) + SUMO
<u>Bacterially expressed RAB1A protein</u>	MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGGMSSMNPEYDYLFKLLIGDSGVGKSCLLRFADDTYTESY ISTIGVDFKIRTIELDGKTIKIQIWDTAGQERFRTITSSYYRGAGHII VVYDVTDQESFNNVKQWLQEI DRYASENVNKLIVGNKCDLTTKKVVDY TTAKEFADSLGIPFLETSAKNATNVEQSFMTMAAEIKKRMGPGATAGG AEKSNVKIQSTPVKQSGGGCC
<u>Native sequence</u>	Amino acids M1 – C205 (end) of human RAB1A. 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: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTT 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
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAACCTCAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA
ACACATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGAATGTCCAGCATGAATCCCGAATAT
GATTATTTATTCAAGTTACTTCTGATTGGCGACTCAGGGGTTGGAAAG
TCTTGCCCTTCTTCTTAGGTTTGCAGATGATACATATACAGAAAGCTAC
ATCAGCACAATTGGTGTGGATTTCAAATAAGA ACTATAGAGTTAGAC
GGGAAAACAATCAAGCTTCAAATATGGGACACAGCAGGCCAGGAAAGA
TTTCGAACAATCACCTCCAGTTATTACAGAGGAGCCCATGGCATCATA
GTTGTGTATGATGTGACAGATCAGGAGTCCTTCAATAATGTTAAACAG
TGGCTGCAGGAAATAGATCGTTATGCCAGTGAAAATGTCAACAAATTG
TTGGTAGGGAACAAATGTGATCTGACCACAAAGAAAGTAGTAGACTAC
ACAACAGCGAAGGAATTTGCTGATTCCCTTGAATTCCGTTTTTGGAA
ACCAGTGCTAAGAATGCAACGAATGTAGAACAGTCTTTCATGACGATG
GCAGCTGAGATTA AAAAGCGAATGGGTCTGGAGCAACAGCTGGTGGT
GCTGAGAAGTCCAATGTTAAAATTCAGAGCACTCCAGTCAAGCAGTCA
GGTGGAGGTTGCTGCTagggcgccgc