

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB35 [1 – 201]

<u>Enzyme description:-</u>	RAB35 [1 – 201]
<u>Clone number:-</u>	DU 26879
<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, 010.77 daltons [After tag cleavage]
Average Mass	23, 025.22 daltons [After tag cleavage]
	[cysteines reduced, methionines have not been oxidised]
<u>Theoretical pI:-</u>	8.53 [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

RAB35 [1 - 201]

<u>Protein</u>	RAB35 [1 - 201]
<u>Clone number</u>	DU 26879
<u>Species</u>	Human
<u>Accession number</u>	NM_001167606.1
<u>Tags</u>	N-terminal His(6) + SUMO
<u>Bacterially expressed RAB35 protein</u>	MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGGMARDYDHLFKLLIIGDSGVGKSSLLRFADNTFSGSYITT IGVDFKIRTVEINGEKVKLQIWDTAGQERFRTITSTYYRGTHGVIVVY DVTSAESFVNVRWLHEINQNCDDVCRIILVGNKNDDPERKVVETEDAY KFAGQMGIQLFETSAKENVNVEEMFNCITELVLRAKKDNLAKQQQQQ NDVVKLTKNSKRKRCC
<u>Native sequence</u>	Amino acids M1 – C201 (end) of human RAB35. 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

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGC AAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATAT TAAACTCAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTG AAAATGACA
ACACATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAACTCACTCAGGTTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGGAATGGCCCGGGACTACGACCACCTC
TTCAAGCTGCTCATCATCGGCGACAGCGGTGTGGGCAAGAGCAGTTTA
CTGTTGCGTTTTTGCAGACAACACTTTCTCAGGCAGCTACATCACCACG
ATCGGAGTGGATTTCAAGATCCGGACCGTGGAGATCAACGGGGAGAAG
GTGAAGCTGCAGATCTGGGACACAGCGGGGCAGGAGCGCTTCCGCACC
ATCACCTCCACGTATTATCGGGGGACCCACGGGGTCATTGTGGTTTTAC
GACGTCACCAGTGCCGAGTCCTTTGTCAACGTCAAGCGGTGGCTTCAC
GAAATCAACCAGA ACTGTGATGATGTGTGCCGAATATTAGTGGGTAAT
AAGAATGACGACCCTGAGCGGAAGGTGGTGGAGACGGAAGATGCCTAC
AAATTCGCCGGGCAGATGGGCATCCAGTTGTTTCGAGACCAGCGCCAAG
GAGAATGTCAACGTGGAAGAGATGTTCAACTGCATCACGGAGCTGGTC
CTCCGAGCAAAGAAAGACAACCTGGCAAAACAGCAGCAGCAACAACAG
AACGATGTGGTGAAGCTCACGAAGAACAGTAAACGAAAGAAACGCTGC
TGCTaagcggccgc