

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB5A [1 – 215]

<u>Enzyme description:-</u>	RAB5A [1 – 215]
<u>Clone number:-</u>	DU 26450
<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, 643.82 daltons [After tag cleavage]
Average Mass	23, 658.68 daltons [After tag cleavage] [cysteines reduced, methionines have not been oxidised]
<u>Theoretical pI:-</u>	8.32 [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

RAB5A [1 - 215]

Protein RAB5A [1 - 215]

Clone number DU 26450

Species Human

Accession number NM_004162.4

Tags N-terminal His(6) + SUMO

Bacterially expressed RAB5A protein
MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMT
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE
VYQEQTGG**MASRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRF**
VKGQFHEFQESTIGAAFLTQTVCLDDTTVKFEIWDTAGQERYHSLAPM
YYRGAQAAIVVYDITNEESFARAKNWVKELQRQASPNIVIALSGNKAD
LANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAKKLPKN
EPQNPANSARGRGVDLTEPTQPTRNQCCSN

Native sequence Amino acids M1 – N215 (end) of human RAB5A.
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 *Eco*R1 sites of pET15b His6-SUMO

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Complete
Nucleotide
Sequence

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA
ACACATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGAATGGCTAGTCGAGGCGCAACAAGA
CCCAACGGGCAAATACTGGAAATAAAATATGCCAGTTCAAACTAGTA
CTTCTGGGAGAGTCCGCTGTTGGCAAATCAAGCCTAGTGCTTCGTTTT
GTGAAAGGCCAATTTTCATGAATTTCAAGAGAGTACCATTGGGGCTGCT
TTTCTAACCCAACTGTATGTCTTGATGACACTACAGTAAAGTTTGAA
ATATGGGATACAGCTGGTCAAGAACGATACCATAGCCTAGCACCAATG
TACTACAGAGGAGCACAAGCAGCCATAGTTGTATATGATATCACAAAT
GAGGAGTCCTTTGCAAGAGCAAAAAATTGGGTTAAAGAACTTCAGAGG
CAAGCAAGTCCTAACATTGTAATAGCTTTATCGGGAAACAAGGCCGAC
CTAGCAAATAAAAGAGCAGTAGATTTCCAGGAAGCACAGTCCTATGCA
GATGACAATAGTTTATTATTCATGGAGACATCCGCTAAAACATCAATG
AATGTAAATGAAATATTCATGGCAATAGCTAAAAAATTGCCAAAGAAT
GAACCACAAAATCCAGGAGCAAATTTCTGCCAGAGGAAGAGGAGTAGAC
CTTACCGAACCACACAACCAACCAGGAATCAGTGTTGTAGTAActaa
gaattc