Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB7L [1 – 203]

Enzyme description:-	RAB7L [1 – 203]
Clone number:-	DU 50291
Source:-	Recombinant
Expression system:-	*E.coli*,
Tag:-	N-terminal His(6) - SUMO
Purification method:-	Ni^{2+}-NTA agarose, Cleavage of His6-SUMO and Gel filtration

Calculated molecular mass:-

Monoisotopic	23, 140.40 daltons [After tag cleavage]
Average Mass	23, 155.25 daltons [After tag cleavage]

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	6.73 [After tag cleavage]
Purity:-	>80 %
Activation Protocol:-	Expressed in the presence of GroEL / GroES

Enzyme storage buffer:-

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

Storage temperature:- -70 °C

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Clone Data Sheet

RAB7L [1 - 203]

Protein	RAB7L [1 - 203]
Clone number	DU 50291
Species	Human
Accession number	NM_003929.2
Tags	N-terminal His(6) + SUMO
Bacterially expressed RAB7L protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGG**MGSRDHLFKVLVVGDAAVGKTSLVQRYSQDSFSKHYKSTV** **GVDFALKVLQWSDYEIVRLQLWDIAGQERFTSMTRLYYRDASACVIMF** **DVTNATTFSNSQRWKQDLDSKLTLPNGEPVPCLLLANKCDLSPWAVSR** **DQIDRFSKENGFTGWTETSVKENKNINEAMRVLIEKMMRNSTEDIMSL** **STQGDYINLQTKSSSWSCC**
Native sequence	Amino acids M1 – C203 (end) of human RAB7L. 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 cleavge of SUMO: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG) residues 9 - 104
Cloning sites	*Bam*H1 and *Not*1 sites of pET15b His6-SUMO

Complete Nucleotide Sequence

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA
ACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGGAATGGGCAGCCGCGACCACCTGTTC
AAAGTGCTGGTGGTGGGGGACGCCGCAGTGGGCAAGACGTCGCTGGTG
CAGCGATATTCCCAGGACAGCTTCAGCAAACACTACAAGTCCACGGTG
GGAGTGGATTTTGCTCTGAAGGTTCTCCAGTGGTCTGACTACGAGATA
GTGCGGCTTCAGCTGTGGGATATTGCAGGGCAGGAGCGCTTCACCTCT
ATGACACGATTGTATTATCGGGATGCCTCTGCCTGTGTTATTATGTTT
GACGTTACCAATGCCACTACCTTCAGCAACAGCCAGAGGTGGAAACAG
GACCTAGACAGCAAGCTCACACTACCCAATGGAGAGCCGGTGCCCTGC
CTGCTCTTGGCCAACAAGTGTGATCTGTCCCCTTGGGCAGTGAGCCGG
GACCAGATTGACCGGTTCAGTAAAGAGAACGGTTTCACAGGTTGGACA
GAAACATCAGTCAAGGAGAACAAAAATATTAATGAGGCTATGAGAGTC
CTCATtgaaaagatgatgagaaattccacagaagatatcatgtctttg
tccacccaaggggactacatcaatctacaaaccaagtcctccagctgg
tcctgctgctag