Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB7B [1 – 199]

Enzyme description:-   RAB7B [1 – 199]

Clone number:-   DU 54145

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   22, 496.63 daltons [After tag cleavage]
Average Mass   22, 510.99 daltons [After tag cleavage]
[cysteines reduced, methionines have not been oxidised

Theoretical pI:-   6.31 [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

Clone Data Sheet

RAB7B [1 - 199]

Protein	RAB7B [1 - 199]
Clone number	DU 54145
Species	Human
Accession number	Q96AH8-1
Tags	N-terminal His(6) + SUMO
Bacterially expressed RAB7B protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGG**SAMNPRKKVDLKLIIVGAIGVGKTSLLHQYVHKTFYEEYQ TTLGASILSKIIILGDTTLKLQIWDTGGQERFRSMVSTFYKGSDGCIL AFDVTDLESFEALDIWRGDVLAKIVPMEQSYPMVLLGNKIDLADRKVP QEVAQGWCREKDIPYFEVSAKNDINVVQAFEMLASRALSRYQSILENH LTESIKLSPDQSRSRCC**
Native sequence	Amino acids M1 – C199 (end) of human RAB7B. Residue M107 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: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG) residues 9 - 104
Cloning sites	*Bam*H1 and *Not*1 sites of pET15b His6-SUMO

Complete
Nucleotide
Sequence

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ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA
ACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAATTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGGGATCCGCCATGAATCCCCGGAAGAAG
GTGGACCTGAAACTCATTATCGTCGGAGCCATTGGTGTGGGAAAGACC
TCCCTCCTTCACCAATATGTGCACAAGACGTTTTATGAGGAATACCAG
ACCACACTGGGGGCCAGCATCCTCTCCAAGATTATCATATTGGGTGAC
ACAACTTTGAAGTTACAGATCTGGGACACGGGCGGTCAGGAGCGGTTC
CGCTCCATGGTGTCCACGTTCTACAAGGGCTCCGATGGCTGCATCCTA
GCTTTTGATGTCACCGACCTGGAGTCTTTTGAAGCCCTGGATATCTGG
CGGGGTGATGTCCTGGCCAAGATTGTCCCCATGGAGCAGTCCTACCCC
ATGGTGTTGTTGGGGAACAAGATCGATCTGGCAGACCGGAAGGTACCC
CAGGAAGTAGCTCAAGGCTGGTGTAGAGAGAAAGATATTCCTTACTTT
GAAGTCAGTGCCAAGAATGACATCAATGTGGTGCAAGCGTTTGAGATG
CTGGCCAGTAGGGCTCTGTCGAGGTACCAGAGCATCTTAGAAAATCAC
CTCACAGAATCCATCAAGCTCTCGCCAGACCAGTCAAGGAGCAGATGC
TGCtga
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