Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB7B [1 – 199 mouse]

Enzyme description:- RAB7B [1 – 199]

Clone number:- DU 68471

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, 487.60 daltons [After tag cleavage]
Average Mass	22, 501.98 daltons [After tag cleavage]

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.83 [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

RAB7B [1 - 199]

Protein	RAB7B [1 - 199]
Clone number	DU 54145
Species	Mouse
Accession number	NM_145509.3
Tags	N-terminal His(6) + SUMO
Bacterially expressed RAB7B protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGG**MNPRKKVDLKLIIVGALGVGKTSLLHQYVHKTFFEEYQTT LGASILSKIIILDDTTLKLQIWDTGGQERFRSMVSTFYKGSDGCILAF DVTDPESFEALDIWRDDVLAKIIPMEQSYPMVVLGNKIDLEDRKVSQE VVHGWCKEKDMPYFEVSAKNDINVVQAFEVLASRALLRYQGTAENHLI DSIKLSPGQPKSRCC**

Native sequence	Amino acids M1 – C199 (end) of mouse RAB7B. 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: (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
GTTTATCAGGAACAAACGGGGGGAATGAATCCCCGAAAGAAAGTGGAC
TTGAAACTTATCATTGTTGGTGCACTTGGCGTGGGAAAGACCTCTCTT
CTTCACCAATATGTACACAAAACATTTTTCGAGGAATACCAGACCACA
CTGGGGGCCAGCATCCTCTCCAAAATCATCATATTGGATGACACAACT
TTGAAGCTGCAGATCTGGGACACAGGTGGTCAGGAGCGGTTCCGCTCA
ATGGTATCAACATTCTACAAAGGTTCCGATGGCTGTATCCTGGCATTT
GATGTTACTGATCCAGAGTCCTTTGAAGCCCTGGATATCTGGAGGGAT
GATGTCCTGGCAAAGATTATCCCTATGGAGCAGTCATATCCCATGGTG
GTGCTGGGGAACAAAATCGATCTGGAAGATAGGAAGGTGTCCCAGGAG
GTGGTCCATGGGTGGTGTAAAGAGAAGGATATGCCATATTTTGAAGTT
AGTGCGAAGAATGACATCAATGTGGTACAAGCCTTTGAGGTTCTGGCC
AGCCGGGCTCTGTTGAGGTACCAGGGCACCGCAGAGAACCACCTCATA
GACTCCATCAAGCTCTCTCCAGGCCAGCCAAAGAGCAGATGCTGCtga