Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB12 [1 – 244]

Enzyme description:-   RAB12 [1 – 244]

Clone number:-   DU 52221

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   27, 231.08 daltons [After tag cleavage]
 Average Mass   27, 248.40 daltons [After tag cleavage]
 [cysteines reduced, methionines have not been oxidised

Theoretical pI:-   8.68 [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

RAB12 [1 - 244]

Protein	RAB12 [1 - 244]
Clone number	DU 52221
Species	Human
Accession number	NM_001025300.2
Tags	N-terminal His(6) + SUMO

Bacterially expressed RAB12 protein

MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE
VYQEQTGG**MDPGAALQRRAGGGGGLGAGSPALSGGQGRRRKQPPRPAD
FKLQVIIIGSRGVGKTSLMERFTDDTFCEACKSTVGVDFKIKTVELRG
KKIRLQIWDTAGQERFNSITSAYYRSAKGIILVYDITKKETFDDLPKW
MKMIDKYASEDAELLLVGNKLDCETDREITRQQGEKFAQQITGMRFCE
ASAKDNFNVDEIFLKLVDDILKKMPLDILRNELSNSILSLQPEPEIPP
ELPPPRPHVRCC**

Native sequence

Amino acids M1 – C244 (end) of human RAB12.
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

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ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA
ACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAATTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGGAATGGACCCGGGCGCCGCGCTGCAG
AGGCGGGCCGGGGGCGGCGGCGGTCTGGGCGCGGGCTCCCCGGCGCTG
TCGGGCGGCCAGGGCCGCCGGAGGAAGCAGCCCCCCAGGCCGGCCGAC
TTCAAGTTGCAGGTCATCATTATCGGCTCCCGCGGCGTGGGCAAGACC
AGCCTGATGGAGCGCTTCACCGACGACACCTTCTGCGAGGCCTGCAAG
TCCACCGTGGGTGTTGACTTCAAAATCAAAACTGTAGAGCTAAGAGGA
AAGAAAATTAGATTACAGATCTGGGACACAGCAGGTCAGGAGAGATTC
AACAGCATTACCTCAGCTTATTACAGAAGTGCCAAGGGGATCATATTA
GTATATGATATCACTAAGAAGGAGACATTTGATGATTTGCCGAAATGG
ATGAAGATGATTGATAAGTATGCTTCAGAAGATGCAGAGCTTCTCTTA
GTTGGAAATAAGTTGGACTGTGAAACGGACAGAGAAATCACCAGGCAG
CAGGGGGAAAAGTTTGCACAGCAGATCACTGGGATGCGGTTCTGTGAA
GCAAGTGCCAAGGATAACTTCAATGTGGACGAGATATTTTTGAAACTT
GTCGATGACATTCTGAAAAAGATGCCTCTGGATATTTTAAGGAATGAG
TTGTCCAATAGTATCCTGTCGTTACAACCAGAGCCTGAGTACCGCCA
GAACTGCCTCCACCAAGACCACATGTCCGATGCTGTtga
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