Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB10 [1 – 200]

Enzyme description:-   RAB10 [1 – 200]

Clone number:-   DU 51062

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, 526.59 daltons [After tag cleavage]
Average Mass   22, 540.93 daltons [After tag cleavage]
[cysteines reduced, methionines have not been oxidised

Theoretical pI:-   8.58 [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

RAB10 [1 - 200]

Protein	RAB10 [1 - 200]
Clone number	DU 51062
Species	Human
Accession number	NM_016131.4
Tags	N-terminal His(6) + SUMO

Bacterially expressed RAB10 protein

MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE
VYQEQTGG**MAKKTYDLLFKLLLIGDSGVGKTCVLFRFSDDAFNTTFIS
TIGIDFKIKTVELQGKKIKLQIWDTAGQERFHTITTSYYRGAMGIMLV
YDITNGKSFENISKWLRNIDEHANEDVERMLLGNKCDMDDKRVVPKGK
GEQIAREHGIRFFETSAKANINIEKAFLTLAEDILRKTPVKEPNSENV
DISSGGGVTGWKSKCC**

Native sequence

Amino acids M1 – C200 (end) of human RAB10.
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

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ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA
ACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGGGtATGGCGAAGAAGACGTACGACCTG
CTTTTCAAGCTGCTCCTGATCGGGGATTCCGGAGTGGGGAAGACCTGC
GTCCTTTTTCGTTTTTCGGATGATGCCTTCAATACTACCTTTATTTCC
ACCATAGGAATAGACTTCAAGATCAAAACAGTTGAATTACAAGGAAAG
AAGATCAAGCTACAGATATGGGATACAGCAGGCCAGGAGCGATTTCAC
ACCATCACAACCTCCTACTACAGAGGCGCAATGGGTATCATGCTAGTA
TATGACATCACCAATGGTAAAAGTTTTGAAAACATCAGCAAATGGCTT
AGAAACATAGATGAGCATGCCAATGAAGATGTGGAAAGAATGTTACTA
GGAAACAAGTGTGATATGGACGACAAAAGAGTTGTACCTAAAGGAAAA
GGAGAACAGATTGCAAGGGAGCATGGTATTAGGTTTTTTGAGACTAGT
GCAAAAGCAAATATAAACATCGAAAAGGCGTTCCTCACGTTAGCTGAA
GATATCCTTCGAAAGACCCCTGTAAAAGAGCCCAACAGTGAAAATGTA
GATATCAGCAGTGGAGGAGGCGTGACAGGCTGGAAGAGCAAATGCTGC
tga
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