Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB32 [1 – 225]

Enzyme description:- RAB32 [1 – 225]

Clone number:- DU 52634

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 25, 068.72 daltons [After tag cleavage]
Average Mass 25, 085.37 daltons [After tag cleavage]
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.08 [After tag cleavage]

Purity:- >80 %

Activation Protocol:- Expressed in the presence of GroEL / GroES

Enzyme storage buffer:-
50 mM HEPES pH 8, 150 mM NaCL,10 µM GDP, 0.5 mM TCEP

Storage temperature:- -70 °C

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Clone Data Sheet

RAB32 [1 - 225]

Protein	RAB32 [1 - 225]
Clone number	DU 52634
Species	Human
Accession number	NM_006834.4
Tags	N-terminal His(6) + SUMO
Bacterially expressed RAB32 protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGGS**MAGGGAGDPGLGAAAAPAPETREHLFKVLVIGELGVGKT** **SIIKRYVHQLFSQHYRATIGVDFALKVLNWDSRTLVRLQLWDIAGQER** **FGNMTRVYYKEAVGAFVVFDISRSSTFEAVLKWKSDLDSKVHLPNGSP** **IPAVLLANKCDQNKDSSQSPSQVDQFCKEHGFAGWFETSAKDNINIEE** **AARFLVEKILVNHQSFPNEENDVDKIKLDQETLRAENKSQCC**
Native sequence	Amino acids M1 – C225 (end) of human RAB32. Residue M106 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 *Xho*1 sites of pET15b His6-SUMO

Complete Nucleotide Sequence

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ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA
ACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAATTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGGATCCATGGCGGGCGGAGGAGCCGGG
GACCCCGGCCTGGGGGCGGCCGCCGCCCCAGCGCCCGAGACCCGCGAG
CACCTCTTCAAGGTGCTGGTGATCGGCGAGCTTGGCGTGGGCAAGACC
AGCATCATCAAGCGCTACGTCCACCAGCTCTTCTCCCAGCACTACCGG
GCCACCATCGGGGTGGACTTCGCCCTCAAGGTCCTCAACTGGGACAGC
AGGACTCTGGTGCGCCTGCAGCTGTGGGACATCGCGGGGCAGGAGCGA
TTTGGCAACATGACCCGAGTATACTACAAGGAAGCTGTTGGTGCTTTT
GTAGTCTTTGATATATCAAGAAGTTCCACATTTGAGGCAGTCTTAAAA
TGGAAAAGTGATCTGGATAGTAAAGTTCATCTTCCAAATGGCAGCCCT
ATCCCTGCTGTCCTCTTGGCTAACAAATGTGACCAGAACAAGGACAGT
AGCCAGAGTCCTTCCCAGGTGGACCAATTCTGCAAAGAACATGGCTTT
GCCGGATGGTTTGAAACCTCTGCAAAGGATAACATAAACATAGAGGAA
GCTGCCCGGTTCCTAGTGGAGAAGATTCTTGTAAACCACCAAAGCTTT
CCTAATGAAGAAAACGATGTGGACAAAATTAAGCTAGATCAAGAGACC
TTGAGAGCAGAGAACAAATCCCAGTGTTGCtga
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