Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB43 [1 – 212]

Enzyme description:- RAB43 [1 – 212]

Clone number:- DU 26431

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal His(6) -SUMO

Purification method:- Ni^{2+}-NTA agarose

Calculated molecular mass:-

Monoisotopic	35, 398.64 daltons
Average Mass	35, 420.86 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.74

Purity:- >80 %

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

RAB43 [1 - 212]

Protein	RAB43 [1 - 212]
Clone number	DU 26431
Species	Human
Accession number	NM_198490.2
Tags	N-terminal His(6) + SUMO
Bacterially expressed protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVQMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGG**MAGPGPGPGDPDEQYDFLFKLVLVGDASVGKTCVVQRFKT GAFSERQGSTIGVDFTMKTLEIQGKRVKLQIWDTAGQERFRTITQSYY RSANGAILAYDITKRSSFLSVPHWIEDVRKYAGSNIVQLLIGNKSDLS ELREVSLAEAQSLAEHYDILCAIETSAKDSSNVEEAFLRVATELIMRH GGPLFSEKSPDHIQLNSKDIGEGWGCGC**
Native sequence	Amino acids M1 – C212 (end) of human RAB43. 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 site of pET15 His-SUMO

Nucleotide Sequence Of Insert:-

ggatccATGGCAGGGCCGGGCCCAGGCCCGGGGGACCCGGACGAGCAGTACGATTTCCT
GTTCAAGCTGGTGCTGGTGGGCGACGCAAGCGTGGGCAAGACGTGCGTGGTGCAGCGCT
TCAAGACCGGCGCCTTCTCGGAGCGCCAGGGAAGCACCATCGGCGTCGACTTCACCATG
AAGACGCTGGAGATCCAGGGCAAGCGGGTCAAGCTGCAGATCTGGGACACGGCCGGCCA
GGAGCGGTTCCGCACCATCACCCAGAGCTACTACCGCAGTGCCAATGGGGCCATCCTTG
CCTACGACATCACCAAGAGGAGCTCCTTCCTGTCGGTGCCTCACTGGATTGAGGATGTG
AGGAAGTATGCGGGCTCCAACATTGTGCAGCTGCTGATCGGGAACAAGTCAGACCTCAG
CGAGCTTCGGGAGGTCTCCTTGGCTGAGGCACAGAGCCTGGCTGAGCACTATGACATCC
TGTGTGCCATTGAGACGTCTGCCAAGGACTCGAGCAACGTGGAGGAGGCCTTCCTGAGG
GTGGCCACGGAGCTCATCATGCGGCACGGGGGCCCCTTGTTCAGCGAGAAGAGCCCCGA
CCACATCCAGCTGAACAGCAAGGACATCGGAGAAGGCTGGGGCTGCGGGTGCtgagcgg
ccgc