

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB8A T72A [1 – 207]

<u>Enzyme description:-</u>	RAB8A T72A [1 – 207]
<u>Clone number:-</u>	DU 47433
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i> ,
<u>Tag:-</u>	N-terminal His(6) - SUMO
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose, Cleavage of His6-SUMO and Gel filtration
<u>Calculated molecular mass:-</u>	
Monoisotopic	23, 623.18 daltons [After tag cleavage]
Average Mass	23, 638.18 daltons [After tag cleavage]
	[cysteines reduced, methionines have not been oxidised]
<u>Theoretical pI:-</u>	9.15 [After tag cleavage]
<u>Purity:-</u>	>80 %
<u>Activation Protocol:-</u>	Expressed in the presence of GroEL / GroES
<u>Enzyme storage buffer:-</u>	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
<u>Storage temperature:-</u>	-70 °C

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Clone Data Sheet

RAB8A T72A [1 - 207]

Protein RAB8A T72A [1 - 207]

Clone number DU 47433

Species Human

Accession number P61006-1

Tags N-terminal His(6) + SUMO

Bacterially expressed RAB8A protein MGHHHHHS DQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTT
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE
VYQEQTGG**MAKTYDYLFKLLIGDSGVGKTCVLF**RFSEDAFNSTF**I**ST
IGIDFKIRTIELDGKRIKLQIWDTAG**QERFRA**ITTAYYRGAMGIMLVY
DITNEKSF DNIRNWIRNIEEHASADVEKMILGNKCDVNDKRQVSKERG
EKLALDYGIKFMETSAKANINVENAFFTLARDIKAKMDKKLEGNSPQG
SNQGVKITPDQ**QKRSSFF**RCVLL

Native sequence Amino acids M1 – L207 (end) of human RAB8A.
Residue M105 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 – 7.

The enzyme has a T72**A** mutation.
Residue T72 is equivalent to **A**176 of the fusion protein.

Protease cleavage SENP1 cleavage of SUMO:
(SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTT
HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME
EEDVIEVYQEQTGG) residues 9 - 104

Cloning sites *Bam*H1 and *Not*1 sites of pET15b His6-SUMO

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**Complete
Nucleotide
Sequence**

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAA
GTCATTGGACAGGATAGCAGTGAGATTCACCTCAAAGTGAAAATGACA
ACACATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGT
CCAATGAATTCACCTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGAATGGCGAAGACCTACGATTACCTG
TTCAAGCTGCTGCTGATCGGGGACTCGGGGGTGGGGAAGACCTGTGTC
CTGTTCCGCTTCTCCGAGGACGCCTTCAACTCCACTTTTATCTCCACC
ATAGGAATTGACTTTAAAATTAGGACCATAGAGCTCGATGGCAAGAGA
ATTAAACTGCAGATATGGGACACAGCCGGTCAGGAACGGTTTCGGGCG
ATCACAACGGCCTACTACAGGGGTGCAATGGGCATCATGCTGGTCTAC
GACATCACCAACGAGAAGTCCTTCGACAACATCCGGAACCTGGATTGCG
AACATTGAGGAGCACGCCTCTGCAGACGTCGAAAAGATGATACTCGGG
AACAAAGTGTGATGTGAATGACAAGAGACAAGTTTCCAAGGAACGGGGA
GAAAAGCTGGCCCTCGACTATGGAATCAAGTTCATGGAGACCAGCGCG
AAGCCAACATCAATGTGGAAAATGCATTTTTTCACTCTCGCCAGAGAT
ATCAAAGCAAAAATGGACAAAAAATTGGAAGGCAACAGCCCCAGGGG
AGCAACCAGGGAGTCAAATCACACCGGACCAGCAGAAGAGGAGCAGC
TTTTTCCGATGTGTTCTTCTGtga