Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RAB32 [1 – 225]

Enzyme description:- RAB32 [1 – 225]

Clone number:- DU 58222

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST - SUMO

Purification method:- GSH-Sepharose, Cleavage of GST-SUMO and Gel filtration

Calculated molecular mass:-
Monoisotopic 24, 981.69 daltons [After tag cleavage]
Average Mass 24, 997.29 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 58222
Species	Human
Accession number	NM_006834.4
Tags	N-terminal GST + SUMO
Bacterially expressed RAB32 protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPSDQEAKPSTEDL GDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNS LRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTGG**MAGGGAGDPGLG** **AAAAPAPETREHLFKVLVIGELGVGKTSIIKRYVHQLFSQHYRATIGV** **DFALKVLNWDSRTLVRLQLWDIAGQERFGNMTRVYYKEAVGAFVVFDI** **SRSSTFEAVLKWKSDLDSKVHLPNGSPIPAVLLANKCDQNKDSSQSPS** **QVDQFCKEHGFAGWFETSAKDNINIEEAARFLVEKILVNHQSFPNEEN** **DVDKIKLDQETLRAENKSQCC**
Native sequence	Amino acids M1 – C225 (end) of human RAB32. Residue M325 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	SENP1 cleavage of SUMO: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG) residues 229 - 324
Cloning sites	*Bam*H1 and *Xho*1 sites of pGex6P-SUMO

Complete Nucleotide Sequence

atgtcccctatactaggttattggaaaattaagggccttgtgcaaccc
actcgacttcttttggaatatcttgaagaaaaatatgaagagcatttg
tatgagcgcgatgaaggtgataaatggcgaaacaaaaagtttgaattg
ggtttggagtttcccaatcttccttattatattgatggtgatgttaaa
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atgttgggtggttgtccaaaagagcgtgcagagatttcaatgcttgaa
ggagcggttttggatattagatacggtgtttcgagaattgcatatagt
aaagactttgaaactctcaaagttgattttcttagcaagctacctgaa
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ggtgatcatgtaacccatcctgacttcatgttgtatgacgctcttgat
gttgttttatacatggacccaatgtgcctggatgcgttcccaaaatta
gtttgttttaaaaaacgtattgaagctatcccacaaattgataagtac
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acgtttggtggtggcgaccatcctccaaaatcggatctggaagttctg
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gatagcagtgagattcacttcaaagtgaaaatgacaacacatctcaag
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ctcaggtttctctttgagggtcagagaattgctgataatcatactcca
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GCGGCCGCCGCCCCAGCGCCCGAGACCCGCGAGCACCTCTTCAAGGTG
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TCAAGAAGTTCCACATTTGAGGCAGTCTTAAAATGGAAAAGTGATCTG
GATAGTAAAGTTCATCTTCCAAATGGCAGCCCTATCCCTGCTGTCCTC
TTGGCTAACAAATGTGACCAGAACAAGGACAGTAGCCAGAGTCCTTCC
CAGGTGGACCAATTCTGCAAAGAACATGGCTTTGCCGGATGGTTTGAA
ACCTCTGCAAAGGATAACATAAACATAGAGGAAGCTGCCCGGTTCCTA
GTGGAGAAGATTCTTGTAAACCACCAAAGCTTTCCTAATGAAGAAAAC
GATGTGGACAAAATTAAGCTAGATCAAGAGACCTTGAGAGCAGAGAAC
AAATCCCAGTGTTGCtga

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