Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of RAB43 [1 – 212]

Enzyme description:- RAB43 [1 – 212]

Clone number:- DU 50329

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 50, 131.38 daltons
Average Mass 50, 163.53 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.60

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

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Clone Data Sheet

RAB43 [1 – 212]

Protein	RAB434 [1 – 212]
Clone number	DU 50329
Species	Human
Accession number	NM_198490.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MAGPGPGPG** **DPDEQYDFLFKLVLVGDASVGKTCVVQRFKTGAFSERQGSTIGVDFTM** **KTLEIQGKRVKLQIWDTAGQERFRTITQSYYRSANGAILAYDITKRSS** **FLSVPHWIEDVRKYAGSNIVQLLIGNKSDLSELREVSLAEAQSLAEHY** **DILCAIETSAKDSSNVEEAFLRVATELIMRHGGPLFSEKSPDHIQLNS** **KDIGEGWGCGC**
Native sequence	Amino acids M1 – C212 (end) of human RAB43. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1 site of pGEX6P-1

Nucleotide Sequence
Of Insert

ggatccATGGCAGGGCCGGGCCCAGGCCCGGGGGACCCGGACGAGCAGTACGATTTCCTGTTCAA
GCTGGTGCTGGTGGGCGACGCAAGCGTGGGCAAGACGTGCGTGGTGCAGCGCTTCAAGACCGGCG
CCTTCTCGGAGCGCCAGGGAAGCACCATCGGCGTCGACTTCACCATGAAGACGCTGGAGATCCAG
GGCAAGCGGGTCAAGCTGCAGATCTGGGACACGGCCGGCCAGGAGCGGTTCCGCACCATCACCCA
GAGCTACTACCGCAGTGCCAATGGGGCCATCCTTGCCTACGACATCACCAAGAGGAGCTCCTTCC
TGTCGGTGCCTCACTGGATTGAGGATGTGAGGAAGTATGCGGGCTCCAACATTGTGCAGCTGCTG
ATCGGGAACAAGTCAGACCTCAGCGAGCTTCGGGAGGTCTCCTTGGCTGAGGCACAGAGCCTGGC
TGAGCACTATGACATCCTGTGTGCCATTGAGACGTCTGCCAAGGACTCGAGCAACGTGGAGGAGG
CCTTCCTGAGGGTGGCCACGGAGCTCATCATGCGGCACGGGGGCCCCTTGTTCAGCGAGAAGAGC
CCCGACCACATCCAGCTGAACAGCAAGGACATCGGAGAAGGCTGGGGCTGCGGGTGCtgagcggc
cgc

Nucleotide sequence of insert

CAGCGCTTCAAGACCGGCGCCTTCTCGGAGCGCCAGGGAAGCACCATCGGCGTCGACT
TCACCATGAAGACGCTGGAGATCCAGGGCAAGCGGGTCAAGCTGCAGATCTGGGACAC
GGCCGGCCAGGAGCGGTTCCGCACCATCACCCAGAGCTACTACCGCAGTGCCAATGGG
GCCATCCTTGCCTACGACATCACCAAGAGGAGCTCCTTCCTGTCGGTGCCTCACTGGAT
TGAGGATGTGAGGAAGTATGCGGGCTCCAACATTGTGCAGCTGCTGATCGGGAACAAG
TCAGACCTCAGCGAGCTTCGGGAGGTCTCCTTGGCTGAGGCACAGAGCCTGGCTGAGC
ACTATGACATCCTGTGTGCCATTGAGACGTCTGCCAAGGACTCGAGCAACGTGGAGGA
GGCCTTCCTGAGGGTGGCCACGGAGCTCATCATGCGGCACGGGGGCCCCTTGTTCAGC
GAGAAGAGCCCCGACCACATCCAGCTGAACAGCAAGGACATCGGAGAAGGCTGGGGCT
GCGGGTGCTGAGCGGCCGC

GGATCCATGG
CAGGGCCGGG
CCCAGGCCCG
GGGGACCCGG
ACGAGCAGTA
CGATTTCCTGT
TCAAGCTGGT
GCTGGTGGGC
GACGCAAGCG
TGGGCAAGAC
GTGCGTGGTG