Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Cullin-3 / Rbx1

Enzyme description:- Cullin-3 / Rbx1 dimer

Clone number:- DU23291

Source:- Recombinant

Tag:- cleaved from Dac-tag

Purification method:- Ampicillin Sepharose, SEC

Expression level:- 3mg/L

Calculated molecular mass:-

Monoisotopic	Cullin-3: 89074 Da
Average Mass	Cullin-3: 89129 Da
Monoisotopic	Rbx1: 12265 Da
Average Mass	Rbx1: 12273 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- Cullin2: 8.98 Rbx1: 7.00

Purity:- 90%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM TCEP

Storage temperature:- -80ºC

Assay:-

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Clone Data Sheet

Protein name

Protein	Cullin-3 1-768 (full length)
Synonyms	Cul3
Clone Number	DU23291
Species	Human
Accession Number	Q13618
Tags	cleaved from Dac-tag
Aminoacid sequence of the purified and cleaved Cullin-3	GGS**MSNLSKGTGSRKDTKMRIRAFPMTMDEKYVNSIWDLLKNAIQEIQ RKNNSGLSFEELYRNAYTMVLHKHGEKLYTGLREVVTEHLINKVREDV LNSLNNNFLQTLNQAWNDHQTAMVMIRDILMYMDRVYVQQNNVENVYN LGLIIFRDQVVRYGCIRDHLRQTLLDMIARERKGEVVDRGAIRNACQM LMILGLEGRSVYEEDFEAPFLEMSAEFFQMESQKFLAENSASVYIKKV EARINEEIERVMHCLDKSTEEPIVKVVERELISKHMKTIVEMENSGLV HMLKNGKTEDLGCMYKLFSRVPNGLKTMCECMSSYLREQGKALVSEEG EGKNPVDYIQGLLDLKSRFDRFLLESFNNDRLFKQTIAGDFEYFLNLN SRSPEYLSLFIDDKLKKGVKGLTEQEVETILDKAMVLFRFMQEKDVFE RYYKQHLARRLLTNKSVSDDSEKNMISKLKTECGCQFTSKLEGMFRDM SISNTTMDEFRQHLQATGVSLGGVDLTVRVLTTGYWPTQSATPKCNIP PAPRHAFEIFRRFYLAKHSGRQLTLQHHMGSADLNATFYGPVKKEDGS EVGVGGAQVTGSNTRKHILQVSTFQMTILMLFNNREKYTFEEIQQETD IPERELVRALQSLACGKPTQRVLTKEPKSKEIENGHIFTVNDQFTSKL HRVKIQTVAAKQGESDPERKETRQKVDDDRKHEIEAAIVRIMKSRKKM QHNVLVAEVTQQLKARFLPSPVVIKKRIEGLIEREYLARTPEDRKVYT YVA**
Native sequence	in bold
Protease cleavage	TEV site underlined
Cloning sites	BamH1 / Not1

DNA sequence of the Cullin 3 insert

GGATCCATGTCTAACCTGTCTAAGGGCACTGGCAGCCGTAAGGATACCAAGAT
GAGAATCAGAGCCTTCCCGATGACTATGGACGAAAAGTACGTGAACTCCATCT
GGGACCTGCTCAAGAACGCTATCCAGGAGATCCAAAGGAAGAACAACTCCGGC
TTGTCTTTCGAGGAACTGTACAGAAACGCCTACACTATGGTGCTGCACAAGCA
CGGCGAGAAGCTCTACACTGGCTTGCGTGAAGTGGTCACAGAGCACCTCATCA
ACAAGGTTCGCGAAGATGTGCTCAACTCTTTGAACAACAACTTCCTGCAGACT
CTCAACCAGGCTTGGAACGACCACCAGACAGCTATGGTCATGATCAGGGATAT
CCTGATGTACATGGACAGAGTCTACGTTCAGCAAAACAACGTGGAGAACGTCT
ACAACCTCGGATTGATCATCTTCCGTGACCAGGTTGTGCGCTACGGTTGCATC
CGTGATCACCTCCGCCAAACCTTGCTGGACATGATCGCTCGTGAACGCAAGGG
AGAGGTCGTTGATAGGGGTGCTATCAGAAACGCCTGTCAGATGCTGATGATCC
TGGGTCTCGAAGGCAGGAGCGTCTACGAGGAAGACTTCGAGGCTCCCTTCCTC
GAAATGTCGGCCGAGTTCTTCCAGATGGAATCCCAAAAGTTCTTGGCTGAGAA
CAGCGCCTCAGTCTACATCAAGAAGGTTGAAGCCCGTATCAACGAGGAAATCG
AGCGCGTTATGCACTGCCTGGACAAGTCAACCGAGGAACCTATCGTCAAGGTG
GTCGAGCGCGAACTCATCTCCAAGCACATGAAGACCATCGTCGAGATGGAAAA
CTCTGGTCTGGTTCACATGCTCAAGAACGGCAAGACTGAGGACCTGGGATGTA
TGTACAAGCTCTTCTCCAGGGTGCCCAACGGCCTGAAGACTATGTGCGAATGT
ATGTCCTCTTACCTCAGAGAGCAGGGAAAGGCTTTGGTTTCTGAGGAAGGCGA
GGGAAAGAACCCTGTGGACTACATCCAAGGCCTCTTGGATTTGAAGTCTAGGT
TCGACCGTTTCCTGCTGGAGTCCTTCAACAACGATAGGCTGTTCAAGCAGACC
ATCGCCGGAGACTTCGAGTACTTCTTGAACCTGAACTCCCGTTCCCCTGAATA
CCTCAGCTTGTTCATCGACGATAAGCTGAAGAAGGGAGTGAAGGGTCTCACCG
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CAGGAAAAGGACGTTTTCGAGCGCTACTACAAGCAACACCTGGCCCGTCGCTT
GCTGACTAACAAGTCTGTGAGCGACGATAGCGAAAAGAACATGATCTCAAAGT
TGAAGACCGAGTGCGGTTGTCAGTTCACTTCTAAGCTGGAAGGCATGTTCCGT
GATATGTCAATCTCGAACACCACTATGGACGAGTTCCGCCAGCACCTGCAAGC
TACCGGTGTGTCACTCGGTGGCGTGGACCTGACTGTGCGTGTCCTGACAACCG
GCTACTGGCCCACTCAGTCGGCTACACCTAAGTGCAACATCCCCCCTGCTCCA
CGTCACGCCTTCGAAATCTTCAGGAGATTCTACTTGGCCAAGCACAGCGGACG
CCAGCTGACACTCCAACACCACATGGGTTCAGCTGATCTGAACGCCACCTTCT
ACGGCCCGGTGAAGAAGGAAGACGGCTCCGAGGTTGGAGTGGGAGGTGCTCAG
GTGACCGGTTCAAACACTCGCAAGCACATCCTGCAGGTCTCGACATTCCAAAT
GACCATCTTGATGCTGTTCAACAACAGGGAGAAGTACACATTCGAGGAAATCC
AGCAAGAAACCGACATCCCAGAACGTGAGCTGGTGCGTGCTCTGCAGTCGCTG
GCTTGCGGAAAGCCAACTCAACGCGTCTTGACAAAGGAGCCGAAGTCCAAGGA
AATCGAGAACGGTCACATCTTCACAGTGAACGATCAGTTCACCTCTAAGCTGC
ACCGTGTTAAGATCCAGACTGTGGCTGCCAAGCAAGGCGAGAGCGACCCCGAA
AGGAAGGAGACAAGACAGAAGGTGGACGATGACCGCAAGCACGAAATCGAGGC
TGCCATCGTCAGGATCATGAAGAGCAGAAAGAAGATGCAACACAACGTCCTCG
TTGCTGAGGTCACCCAGCAACTCAAGGCCAGGTTCTTGCCATCACCGGTTGTG
ATCAAGAAGAGAATCGAGGGTCTGATCGAGAGAGAATACTTGGCTAGGACTCC
AGAGGACAGAAAGGTTTACACTTACGTTGCTTAAGCGGCCGC

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Clone Data Sheet

Protein name

Protein	Rbx1 1-108 (full length)
Synonyms	RNF75, ROC1
Clone Number	DU23263
Species	Human
Accession Number	P62877
Tags	N/A
Aminoacid sequence of the purified Rbx1	MAAAMDVDTPSGTNSGAGKKRFEVKKWNAVALWAWDIVVDNCAICRNH IMDLCIECQANQASATSEECTVAWGVCNHAFHFHCISRWLKTRQVCPL DNREWEFQKYGH
Native sequence	in bold
Protease cleavage	N/A
Cloning sites	Nhe1 / Kpn1
DNA sequence of the Rbx1 insert	gctagcATGGCGGCAGCGATGGATGTGGATACCCCGAGCGGCACCAAC AGCGGCGCGGGCAAGAAGCGCTTTGAAGTGAAAAAGTGGAATGCAGTA GCCCTCTGGGCCTGGGATATTGTGGTTGATAACTGTGCCATCTGCAGG AACCACATTATGGATCTTTGCATAGAATGTCAAGCTAACCAGGCGTCC GCTACTTCAGAAGAGTGTACTGTCGCATGGGGAGTCTGTAACCATGCT TTTCACTTCCACTGCATCTCTCGCTGGCTCAAAACACGACAGGTGTGT CCATTGGACAACAGAGAGTGGGAATTCCAAAAGTATGGGCACTAGggt acc