

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Cullin-3 delta 403-459/ Rbx1

Enzyme description:- Cullin-3 delta 403-459/ Rbx1 dimer

Clone number:- DU23292

Source:- Recombinant

Tag:- cleaved from Dac-tag

Purification method:- Ampicillin Sepharose

Expression level:- 3mg/L

Calculated molecular mass:-

Monoisotopic	Cullin-3: 82000 Da
Average Mass	Cullin-3: 82051 Da
Monoisotopic	Rbx1: 12265 Da
Average Mass	Rbx1: 12273 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- Cullin 3: 8.95 Rbx1: 7.00

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80°C

Assay:-

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Clone Data Sheet

Protein name

Protein Cullin-3 1-768 delta 403-459 (full length)

Synonyms Cul3

Clone Number DU23292

Species Human

Accession Number Q13618

Tags cleaved from Dac-tag

Aminoacid sequence of
the purified and cleaved
Cullin-3

**GGSMSNLSKGTGSRKDTKMRIRAFPMDEKYVNSIWLLKNAIQEIQ
RKNNSGLSFEELYRNAYTMVLHKKHGEKLYTGLREVVTEHLINKVREDV
LNSLNNNFLOTLNQAWNDHQAMVMIRDILMYMDRVYVQONNVENVYN
LGLIIFRDQVVRGYCIRDHLRQTLDDMIARERKGEVVDRGAIRNACQM
LMILGLEGRSVYEEDFEAPFLEMSAEFFQMESQKFLAENSASVYIKKV
EARINEEIERVMHCLDKSTEPIVKVVERELISKHMKTIVEMENSGLV
HMLKNGKTEDLGCMYKLF SRVPNGLKTMCECMSSYLREQKALVSEEG
EGKNPVDYIQGLLDLKSFRDRFLLESFNNDRLFQTIAGDFEYFLNLN
SRSPEYLSLFIIDDKLKKGVKGTECGCQFTSKLEGFRDMSISNTTMD
FRQHLQATGVS LGVDLTVRVLTGYPWPTQSATPKCNIPAPRHAFEI
FRRFYLAKHSGRQLTLQHMG SADLNATFYGPVKKEDGSEVGVGGAQV
TGSNTRKHILQVSTFQMTILMLFNNREKYTFEEIQQETDIPERELVRA
LQSLACGKPTQRVLTKEPKSKEIENGHIFTVNDQFTSKLHRVKIQTVA
AKQGESDPERKETRQKVDDDRKHEIEAAIVRIMKSRKKMQHNVLVAEV
TQQLKARFLPSPVVIKKRIEGLIEREYLARTPEDRKVYTYVA**

Native sequence in bold

Protease cleavage TEV site underlined

Cloning sites BamH1 / Not1

**DNA sequence
of the Cullin 3
insert**

GGATCCATGTCTAACCTGTCTAAGGGCACTGGCAGCCGTAAGGATACCAAGAT
GAGAATCAGAGCCTTCCCGATGACTATGGACGAAAAGTACGTGAACTCCATCT
GGGACCTGCTCAAGAACGCTATCCAGGAGATCCAAAGGAAGAACAACCTCCGGC
TTGTCTTTCGAGGAAGTGTACAGAAACGCCTACACTATGGTGTGCACAAGCA
CGGCGAGAAGCTCTACACTGGCTTGCCTGAAGTGGTCACAGAGCACCTCATCA
ACAAGGTTTCGCGAAGATGTGCTCAACTCTTTGAACAACAACCTTCCTGCAGACT
CTCAACCAGGCTTGAACGACCACCAGACAGCTATGGTCATGATCAGGGATAT
CCTGATGTACATGGACAGAGTCTACGTTTCAGCAAAAACAACGTGGAGAACGTCT
ACAACCTCGGATTGATCATCTTCCGTGACCAGGTTGTGCGCTACGGTTGCATC
CGTGATCACCTCCGCCAAACCTTGTGGACATGATCGCTCGTGAACGCAAGGG
AGAGGTCGTTGATAGGGGTGCTATCAGAAACGCCTGTGATGCTGATGATCC
TGGGTCTCGAAGGCAGGAGCGTCTACGAGGAAGACTTCGAGGCTCCCTTCCCTC
GAAATGTCGGCCGAGTCTTCCAGATGGAATCCCAAAGTTCTTGGCTGAGAA
CAGCGCTCAGTCTACATCAAGAAGGTTGAAGCCCGTATCAACGAGGAAATCG
AGCGCGTTATGCACTGCCGACAAAGTCAACCGAGGAACCTATCGTCAAGGTG
GTCGAGCGCAACTCATCTCCAAGCACATGAAGACCATCGTCGAGATGGAAAA
CTCTGGTCTGGTTCACATGCTCAAGAACGGCAAGACTGAGGACCTGGGATGTA
TGTACAAGCTCTTCTCCAGGGTGCCCAACGGCCTGAAGACTATGTGCGAATGT
ATGTCCTCTTACCTCAGAGAGCAGGGAAAGGCTTTGGTTTCTGAGGAAGGCGA
GGGAAAGAACCCTGTGGACTACATCCAAGGCCTCTTGGATTTGAAGTCTAGGT
TCGACCGTTTCCCTGCTGGAGTCTTCAACAACGATAGGCTGTTCAAGCAGACC
ATCGCCGGAGACTTCGAGTACTTCTTGAACCTGAACTCCCGTTCCCCTGAATA
CCTCAGCTTGTTCATCGACGATAAGCTGAAGAAGGGAGTGAAGGGTACCGAGT
GCGGTTGTCAGTTCACTTCTAAGCTGGAAGGCATGTTCCGTGATATGTCAATC
TCGAACACCACTATGGACGAGTTCGCGCCAGCACCTGCAAGCTACCGGTGTGTC
ACTCGGTGGCGTGGACCTGACTGTGCGTGTCTGACAACCGGCTACTGGCCCA
CTCAGTCGGCTACACCTAAGTGCAACATCCCCCTGCTCCACGTCACGCCTTC
GAAATCTTCAGGAGATTCCTACTTGGCCAAGCACAGCGGACGCCAGCTGACACT
CCAACACCACATGGGTTTCAGCTGATCTGAACGCCACCTTCTACGGCCCCGGTGA
AGAAGGAAGACGGCTCCGAGGTTGGAGTGGGAGGTGCTCAGGTGACCGGTTCA
AACACTCGCAAGCACATCTGCAGGTCCTCGACATTCCAAATGACCATCTTGAT
GCTGTTCAACAACAGGGAGAAGTACACATTCGAGGAAATCCAGCAAGAAACCG
ACATCCCAGAACGTGAGCTGGTGCCTGCTCTGCAGTCGCTGGCTTGCAGGAAAG
CCAACCTCAACGCGTCTTGACAAAGGAGCCGAAGTCCAAGGAAATCGAGAACGG
TCACATCTTACAGTGAACGATCAGTTCACCTCTAAGCTGCACCGTGTAAAGA
TCCAGACTGTGGCTGCCAAGCAAGGCGAGAGCGACCCCGAAAGGAAGGAGACA
AGACAGAAGGTGGACGATGACCGCAAGCACGAAATCGAGGCTGCCATCGTCAG
GATCATGAAGAGCAGAAAAGAAGATGCAACACAACGTCCTCGTTGCTGAGGTCA
CCCAGCAACTCAAGGCCAGGTTCTTGCCATCACCGGTTGTGATCAAGAAGAGA
ATCGAGGTCGATCGAGAGAGAATACTTGGCTAGGACTCCAGAGGACAGAAA
GGTTTACACTTACGTTGCTTAAGCGGCCGC

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Clone Data Sheet

Protein name

<u>Protein</u>	Rbx1 1-108 (full length)
<u>Synonyms</u>	RNF75, ROC1
<u>Clone Number</u>	DU23263
<u>Species</u>	Human
<u>Accession Number</u>	P62877
<u>Tags</u>	N/A
Aminoacid sequence of the purified Rbx1	MAAAMDVDTPSGTNSGAGKKRFEVKKWNAVALWAWDIIVVDNCAICRNH IMDLCIECQANQASATSEECTVAWGVCNHAFHFHCISRWLKTRQVCPL DNREWEFQKYGH
Native sequence	in bold
Protease cleavage	N/A
Cloning sites	Nhe1 / Kpn1
DNA sequence of the Rbx1 insert	gctagc ATGGCGGCAGCGATGGATGTGGATACCCGAGCGGCACCAAC AGCGGCGCGGGCAAGAAGCGCTTTGAAGTGAAAAAGTGGAAATGCAGTA GCCCTCTGGGCCTGGGATATTGTGGTTGATAACTGTGCCATCTGCAGG AACCACATTATGGATCTTTGCATAGAATGTCAAGCTAACCAGGCGTCC GCTACTTCAGAAGAGTGTACTGTTCGCATGGGGAGTCTGTAACCATGCT TTTCACTTCCACTGCATCTCTCGCTGGCTCAAAAACACGACAGGTGTGT CCATTGGACAACAGAGAGTGGGAATTCCAAAAGTATGGGCACTAG ggt acc