Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Cullin-2 / Rbx1

Enzyme description:- Cullin-2 / Rbx1 dimer

Clone number:- DU23263

Source:- Recombinant

Tag:- cleaved from Dac-tag

Purification method:- Ampicillin Sepharose, SEC

Expression level:- 3mg/L

Calculated molecular mass:-

Monoisotopic	Cullin-2: 87127 Da
Average Mass	Cullin-2: 87181 Da
Monoisotopic	Rbx1: 12265 Da
Average Mass	Rbx1: 12273 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- Cullin2: 6.82 Rbx1: 7.00

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80ºC

Assay:-

Division of Signal Transduction Therapy

Clone Data Sheet

Protein name

Protein	Cullin-2 1-745 (full length)
Synonyms	Cul2
Clone Number	DU23263
Species	Human
Accession Number	Q13617
Tags	cleaved from Dac-tag
Aminoacid sequence of the purified and cleaved Cullin2	GGS**MSLKPRVVDFDETWNKLLTTIKAVVMLEYVERATWNDRFSDIYAL CVAYPEPLGERLYTETKIFLENHVRHLHKRVLESEEQVLVMYHRYWEE YSKGADYMDCLYRYLNTQFIKKNKLTEADLQYGYGGVDMNEPLMEIGE LALDMWRKLMVEPLQAILIRMLLREIKNDRGGEDPNQKVIHGVINSFV HVEQYKKKFPLKFYQEIFESPFLTETGEYYKQEASNLLQESNCSQYME KVLGRLKDEEIRCRKYLHPSSYTKVIHECQQRMVADHLQFLHAECHNI IRQEKKNDMANMYVLLRAVSTGLPHMIQELQNHIHDEGLRATSNLTQE NMPTLFVESVLEVHGKFVQLINTVLNGDQHFMSALDKALTSVVNYREP KSVCKAPELLAKYCDNLLKKSAKGMTENEVEDRLTSFITVFKYIDDKD VFQKFYARMLAKRLIHGLSMSMDSEEAMINKLKQACGYEFTSKLHRMY TDMSVSADLNNKFNNFIKNQDTVIDLGISFQIYVLQAGAWPLTQAPSS TFAIPQELEKSVQMFELFYSQHFSGRKLTWLHYLCTGEVKMNYLGKPY VAMVTTYQMAVLLAFNNSETVSYKELQDSTQMNEKELTKTIKSLLDVK MINHDSEKEDIDAESSFSLNMNFSSKRTKFKITTSMQKDTPQEMEQTR SAVDEDRKMYLQAAIVRIMKARKVLRHNALIQEVISQSRARFNPSISM IKKCIEVLIDKQYIERSQASADEYSYVA**
Native sequence	in bold
Protease cleavage	TEV site underlined
Cloning sites	BamH1 / Not1

DNA sequence of the Cullin 2 insert

GGATCCATGTCTTTGAAACCAAGAGTAGTAGATTTTGATGAAACATGGAACAA
ACTTTTGACGACAATAAAAGCCGTGGTCATGTTGGAATACGTCGAAAGAGCAA
CATGGAATGACCGTTTCTCAGATATCTATGCTTTATGTGTGGCCTATCCTGAA
CCCCTTGGAGAAAGACTTTATACAGAAACTAAGATTTTTTTGGAAAATCATGT
TCGGCATTTGCATAAGAGAGTTTTGGAGTCAGAAGAACAAGTACTTGTTATGT
ATCATAGGTACTGGGAAGAATACAGCAAGGGTGCAGACTATATGGACTGCTTA
TATAGGTATCTCAACACCCAGTTTATTAAAAAGAATAAATTAACAGAAGCGGA
CCTTCAGTATGGCTATGGTGGTGTAGATATGAATGAACCACTTATGGAAATAG
GAGAGCTAGCATTGGATATGTGGAGGAAATTGATGGTTGAACCACTTCAGGCC
ATCCTTATCCGAATGCTGCTCCGAGAAATCAAAAATGATCGTGGTGGAGAAGA
CCCAAACCAGAAAGTAATCCATGGGGTTATTAACTCCTTTGTTCATGTTGAAC
AGTATAAGAAAAAATTCCCCTTAAAGTTTTATCAGGAAATTTTTGAGTCTCCC
TTTCTGACTGAAACAGGAGAGTATTACAAACAAGAAGCTTCAAATTTATTACA
AGAATCAAACTGCTCACAGTATATGGAAAAGGTTCTAGGTAGATTAAAAGATG
AAGAAATTCGATGTCGAAAATACCTACATCCAAGTTCATATACTAAGGTGATT
CATGAATGTCAACAACGAATGGTAGCAGACCACTTACAGTTTTTACATGCAGA
ATGTCATAATATAATTCGACAAGAGAAAAAAAATGACATGGCAAATATGTACG
TCTTACTCCGTGCTGTGTCCACTGGTTTACCTCATATGATTCAGGAGCTGCAA
AACCACATCCATGATGAGGGCCTTCGAGCAACCAGCAACCTTACTCAGGAAAA
CATGCCAACACTATTTGTGGAGTCAGTTTTGGAAGTGCATGGTAAATTTGTTC
AGCTTATCAACACTGTTTTGAATGGTGATCAGCATTTTATGAGTGCGTTGGAT
AAGGCCCTTACGTCAGTTGTAAATTACAGAGAACCTAAGTCTGTTTGCAAAGC
ACCTGAACTGCTTGCTAAGTACTGTGACAACTTACTGAAGAAGTCAGCGAAAG
GGATGACAGAGAATGAAGTGGAAGACAGGCTCACGAGCTTCATCACAGTGTTC
AAATACATTGATGACAAGGACGTCTTTCAAAAGTTCTACGCAAGAATGCTGGC
AAAACGTTTAATTCATGGGTTATCCATGTCTATGGACTCTGAAGAAGCCATGA
TCAACAAATTAAAGCAAGCCTGTGGTTATGAGTTTACCAGCAAGCTACATCGG
ATGTATACAGATATGAGTGTCAGCGCTGATCTCAACAATAAGTTCAACAATTT
TATCAAAAACCAAGACACAGTAATAGATTTGGGAATTAGTTTTCAAATATATG
TTCTACAGGCTGGTGCGTGGCCTCTTACTCAGGCTCCTTCATCTACGTTTGCA
ATTCCCCAGGAATTAGAAAAAAGTGTACAGATGTTTGAATTATTTTATAGCCA
ACATTTCAGTGGAAGGAAACTTACATGGTTACATTATCTGTGTACAGGTGAAG
TTAAAATGAACTATTTGGGCAAACCATATGTAGCCATGGTTACAACATACCAA
ATGGCAGTTCTTCTTGCCTTTAACAACAGTGAAACTGTCAGTTATAAAGAGCT
TCAGGACAGCACTCAGATGAATGAAAAGGAACTGACAAAAACAATCAAATCAT
TACTTGATGTGAAAATGATTAACCATGATTCAGAAAAGGAAGATATTGATGCA
GAATCTTCGTTTTCATTAAATATGAACTTTAGCAGTAAAAGAACAAAATTTAA
AATTACTACATCAATGCAGAAAGACACACCACAAGAAATGGAGCAGACTAGAA
GTGCAGTTGATGAGGACCGGAAAATGTATCTCCAAGCTGCTATAGTTCGTATC
ATGAAAGCACGAAAAGTGCTTCGGCACAATGCCCTTATTCAAGAGGTGATTAG
CCAGTCAAGAGCTAGGTTTAATCCCAGTATCAGCATGATTAAGAAGTGTATTG
AAGTTCTGATAGACAAACAATACATAGAACGCAGCCAGGCGTCGGCAGATGAA
TACAGCTACGTCGCGTGAGCGGCCGC

Division of Signal Transduction Therapy

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