

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Cullin-4a / Rbx1

<u>Enzyme description:-</u>	Cullin-4a / Rbx1 dimer
<u>Clone number:-</u>	DU23656
<u>Source:-</u>	Recombinant
<u>Tag:-</u>	cleaved from Dac-tag
<u>Purification method:-</u>	Ampicillin Sepharose, SEC
<u>Expression level:-</u>	0.5mg/L

Calculated molecular mass:-

Monoisotopic	Cullin-3:	87825 Da
Average Mass	Cullin-3:	87879 Da
Monoisotopic	Rbx1:	12265 Da
Average Mass	Rbx1:	12273 Da

[cysteines reduced, methionines have not been oxidised]

<u>Theoretical pI:-</u>	Cullin2: 8.70	Rbx1: 7.00
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<u>Purity:-</u>	>80%
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Enzyme storage buffer:-

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

<u>Storage temperature:-</u>	-80°C
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Assay:-

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Clone Data Sheet

Protein name

Protein Cullin-4a 1-759 (full length)

Synonyms Cul4a

Clone Number DU23656

Species Human

Accession Number Q13619

Tags cleaved from Dac-tag

Aminoacid sequence of
the purified and cleaved
Cullin-3

**GGSMAD EAPR KGSF SALVGR TNGLT KPAALAAAPAKPGGAGGSKKLVI
KNFRDRPRLPDNYTQDTWRKLHEAVRAVQSSTSI RYNLEELYQAVENL
CSHKVSPMLYKQLRQACEDHVQAQILPFREDSLDSVFLK KINTCWQD
HCRQMIMIRSI FLFLDR TYVLQNSTLPSI WDMGLELFRTHI ISDKMVQ
SKTIDGILLIERERSGEAVDRSLLRSLLGMLS DLQVYKDSFELKFL E
ETNCLYAAEGQRLMQEREVPEYLNHVSKRLEEEGDRVITYLDHSTQKP
LIACVEKQLLGEHLTAILOKGLDHLLENRVPD LAQMYQLFSRVRGGQ
QALLQHWSEYIKTFGTAI VINPEKDKDMVQDLLDFKDKVDHVIEVCFQ
KNERFVNLMKESFETF INKRPNKPAELIAKHVDSKLRAGNKEATDEEL
ERTLDKIMILFRFIHGKDVFEAFYKKDLAKRLLV GKSASVDAEKSMLS
KLEKHECGAAFTSKLEGMFKDMELSKDIMVHFQOHMQNSDSGPIDLTV
NILTMGYWPTYTPMEVHLTPEMI KLOEVFKAFYLGKHSGRKLOWQTTL
GHA VLKAEFKEGKKEFQVSLFQTLVLLMFNEGDGFSFEEIKMATGIED
SELRRTLQSLACGKARVLIKSPKGKEVEDGDKFIFNGEFKHKLFRIKI
NQIQMKETVEEQVSTTERV FQDRQYQIDAAIVRIMKMRKTLGHNLLVS
ELYNQLKFPVKPGDLKKRIESLIDRDYMERDKDNP NQYHYVA**

Native sequence in bold

Protease cleavage TEV site

Cloning sites BamH1 / NotI

**DNA sequence
of the Cullin 4a
insert**

GGATCCATGGCGGACGAGGCCCGCGGAAGGGCAGCTTCTCGGCGCTCGTGGG
CCGCACCAACGGCCTCACCAAGCCCGCGGCCCTGGCCGCGCGCCCGCCAAGC
CGGGGGGCGCGGGCGGCTCCAAGAAGCTGGTCATCAAGAACTTCCGAGACAGA
CCTCGGCTGCCCCACAACCTACACGCAGGACACGTGGCGGAAGCTGCACGAGGC
GGTGCGGGCCGTGCAGAGCAGCACCTCCATCAGGTACAACCTCGAGGAGCTCT
ACCAGGCTGTGGAAAATCTCTGTTCTCACAAAGTCTCCCCAATGCTCTACAAG
CAACTGCGTCAGGCCTGTGAAGACCACGTCCAGGCACAGATCCTTCCGTTTAG
AGAAGACTCACTAGATAGTGTATTTTATTTTTAAAGAAGATTAACACGTGCTGGC
AGGACCCTGCAGACAAATGATCATGATCAGAAGCATCTTCCCTGTTCTTGGAC
CGCACCTATGTGCTGCAGAACTCCACGCTGCCCTCCATCTGGGATATGGGATT
AGAAGTGTTAGAACCCATATTATTAGTGATAAAATGGTTTCAGAGTAAACCA
TTGATGGAATCCTACTGCTGATCGAGCGCGAGAGGAGCGGCGAGGCCGTGGAC
CGGAGCCTGTTGCGGAGCCTCCTGGGCATGCTGTCTGACCTGCAGGTGTATAA
AGATTCATTTGAACTGAAATTTTTGGAAGAGACTAATTGCTTATATGCTGCCG
AAGGCCAAAGGTTAATGCAGGAAAGAGAGGTTCCAGAATATCTTAACCATGTA
AGTAAACGCTTAGAGGAAGAGGGAGACAGAGTAATCACTTACTTGGACCACAG
CACACAGAAACCACTGATTGCTTGTGTGGAGAAACAGCTATTAGGAGAACATT
TAACAGCAATTCTGCAGAAAGGGCTCGACCACTTACTGGATGAGAACAGAGTG
CCGGACCTCGCACAGATGTACCAGCTGTTTCAGCCGGGTGAGGGCGGGCAGCA
GGCGCTGCTGCAGCACTGGAGCGAGTACATCAAGACTTTTGGAACAGCGATCG
TAATCAATCCTGAGAAAGACAAAGACATGGTCCAAGACCTGTTGGACTTCAAG
GACAAGGTGGACCACGTGATCGAGGTCGCTTCCAGAAGAATGAGCGGTTTCGT
CAACCTGATGAAGGAGTCCTTTGAGACGTTTCATCAACAAGAGACCCAACAAGC
CTGCAGAACTGATCGCAAAGCATGTGGATTCAAAGTAAAGAGCAGGCAACAAA
GAAGCCACAGACGAGGAGCTGGAGCGGACGTTGGACAAGATCATGATCCTGTT
CAGGTTTATCCACGGTAAAGATGTCTTTGAAGCATTTTATAAAAAAGATTTGG
CAAAAAGACTCCTTGTGGGAAAAGTGCCTCAGTCGATGCTGAAAAGTCTATG
TTGTCAAAGCTCAAGCATGAGTGCGGTGCAGCCTTACCAGCAAGCTGGAAGG
CATGTTCAAGGACATGGAGCTTTCGAAGGACATCATGGTTTCATTTCAAGCAGC
ATATGCAGAATCAGAGTGACTCAGGCCCTATAGACCTCACAGTGAACATACTC
ACAATGGGCTACTGGCCAACATACAGCCCATGGAAGTGCACCTTAACCCCAAG
AATGATTAAACTTCAGGAAGTATTTAAGGCATTTTATCTTGGAAAAGCACAGTG
GTGCAAAACTTCAGTGGCAAACCTACTTTGGGACATGCTGTTTTAAAGCAGGAG
TTTTAAAGAAGGGAAGAAGGAATTCAGGTGTCCCTCTTCCAGACACTGGTGCT
CCTCATGTTCAACGAGGGAGATGGCTTTCAGCTTTGAGGAGATAAAAATGGCCA
CGGGGATAGAGGATAGTGAATTGCGCAGAACGCTGCAGTCCCTGGCCTGTGGC
AAAGCACGTGTGCTGATTAAAAGTCCCAAAGGAAAAGGAAGTGGAAAGATGGAGA
CAAGTTCATTTTTAATGGAGAGTTCAAGCACAAGTTGTTTAGAATAAAGATCA
ATCAAATTCAGATGAAGGAACTGTTGAGGAACAGGTTAGCACCCTGAGAGA
GTGTTTCAGGATAGACAATATCAGATTGATGCTGCTATCGTCAGAATAATGAA
GATGAGAAAAGACTCTTGGTCATAATCTTCTAGTTTCTGAATTATATAATCAGC
TGAAATTTCCAGTAAAGCCTGGAGATTTGAAAAAGAGAATTGAATCTCTGATA
GACAGAGACTATATGGAGAGAGACAAAGACAATCCGAATCAGTACCACTACGT
GCCTGAGCGGCCGC

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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 IMDLCEIQANQASATSEECTVAWGVCNHAFHFHCISRWLKTRQVCPL 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 TTTCACTTCCACTGCATCTCTCGCTGGCTCAAAACACGACAGGTGTGT CCATTGGACAACAGAGAGTGGGAATTCCAAAAGTATGGGCACTAG ggt acc