

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Cullin-1 / Rbx1

Enzyme description:- Cullin1 (1-776) / Rbx1 (1-108) dimer

Clone number:- DU23290

Source:- Recombinant

Tag:- cleaved from Dac-tag

Purification method:- Ampicillin Sepharose, SEC

Expression level:- 2mg/L

Calculated molecular mass:-

| | | |
|--------------|----------|----------|
| Monoisotopic | Cullin1: | 89823 Da |
| Average Mass | Cullin1: | 89877 Da |
| Monoisotopic | Rbx1: | 12265 Da |
| Average Mass | Rbx1: | 12273 Da |

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- Cullin1: 8.47 Rbx1: 7.00

Purity:- 80%

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-1 1-776 (full length)

Synonyms Cull1

Clone Number DU23290

Species Human

Accession Number Q13616

Tags cleaved from Dac-tag

Aminoacid sequence of
the purified and cleaved
Cullin1

GGSMSSTRSQNP~~HLKQ~~IGLDQIWD~~DLRAGIQQ~~VYTRQSM~~AKSR~~YME~~L~~
YTHVYNYCTSVHQS~~NQ~~ARGAGVPPSKS~~KKGQ~~TPGGAQ~~FV~~GLELYKRLK
EFLK~~NYL~~TNLLKDGEDLMDES~~V~~LKFYTQ~~Q~~WEDYRFSSK~~V~~LNGICAYLN
RHWV~~R~~RECDEGRKGIYEIYSLALVTWRDCLFRPLNKQVTNAVLK~~L~~IEK
ERNGETIN~~TRL~~ISGVVQSYVELGLNEDDAFAK~~GPTL~~TVYKESFESQ~~F~~L
ADTERFY~~T~~RESTEF~~L~~QONPVTEYMKKAEARLLEE~~Q~~RRVQVYLHES~~T~~QD
ELARKCEQ~~V~~LIEKHLEIFHTEFQ~~N~~LLDADKNEDLGRMYNLVSRIQ~~D~~GL
GELKKLLETHIHNOGLAAIEKCGEALNDPKMYVQ~~T~~VLDVHKKYNALV
MSAF~~N~~NDAGFVAALDKACGRFINNNAVTKMAQSSSK~~S~~PELLARYCDSL
LKKSSKNPEEALEDTLNQVMVVF~~K~~YIEDKDV~~F~~QKFYAKMLAKRLV~~H~~Q
NSASDDAEASMI~~S~~KLKQACGF~~E~~YTSKLQ~~R~~MFQDIGVSKDLNEQ~~F~~KKHL
TNSEPLD~~L~~DFSIQVLS~~S~~SGSWPFQ~~Q~~SCTFALPSE~~L~~ERSYQ~~R~~F~~T~~AFYASR
HSGR~~K~~L~~T~~WLYQ~~L~~SKGELVTNCFKNRY~~T~~LQASTFQ~~M~~AILLOYN~~T~~EDAYT
VQ~~L~~TDSTQIKMDILAQV~~L~~QILLKSKLLVLEDENANVDEVELK~~P~~DTLI
KLYLGYKNKLRVNI~~N~~VPMKTEQ~~K~~QEQETTHK~~N~~IEEDR~~K~~LLIQAAIVR
IMKMRKVLKHQ~~L~~LGEVLTQ~~L~~SSRFKPRVPVIKKCIDILIEKEYLERV
DGEKDTYSYLA

Native sequence in bold

Protease cleavage TEV site underlined

Cloning sites BamH1 / Not1

**DNA sequence
of the Dac-
Cullin 1
expression
cassette**

ATGTCTGCAATCCCGGGTGTACCGCAGATCGATGCGGAGTCCTACATCCTGAT
TGACTATAACTCCGGCAAAGTGCTCGCCGAACAGAACGCAGATGTCCGCCGCG
ATCCTGCCAGCCTGACCAAAATGATGACCAGTTACGTTATCGGCCAGGCAATG
AAAGCCGGTAAATTTAAAGAACTGATTTAGTCACTATCGGCAACGACGCATG
GGCCACCGGTAACCCGGTGTTTAAAGGTTCTTCGCTGATGTTCCCTCAAACCGG
GCATGCAGGTTCCGGTTTCTCAGCTGATCCGCGGTATTAACCTGCAATCGGGT
AACGATGCTTGTGTGCGCCATGGCCGATTTTGCCGCTGGTAGCCAGGACGCTTT
TGTTGGCTTGATGAACAGCTACGTTAACGCACTGGGCCTGAAAAATACCCACT
TCCAGACGGTACATGGTCTGGATGCTGATGGTCAGTACAGCTCCGCGCGAGAT
ATGGCGCTGATCGGCCAGGCATTGATCCGTGACGTACCGAATGAATACTCGAT
CTATAAAGAAAAAGAATTTACGTTTAAACGGTATTCGCCAGCTGAACCGTAACG
GCCTGTTATGGGATAACAGCCTGAATGTCGACGGCATCAAACCGGACACACT
GACAAAGCAGGTTACAACCTTGTTGCTTCTGCGACTGAAGGCCAGATGCGCTT
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tcatgaagatgaggaaggttctgaaacaccagcagttacttggcgaggtcctc
actcagctgtcctccaggttcaaacctcgagtcctctgtgatcaagaaatgcat
tgacattctaattgagaaagaatatttggagcgcagtggtggtgaaaggaca
cctacagttacttggcttaa

Division of Signal Transduction Therapy

Clone Data Sheet

Protein name

| | |
|--|---|
| <u>Protein</u> | Rbx1 1-108 (full length) |
| <u>Synonyms</u> | RNF75, ROC1 |
| <u>Clone Number</u> | DU23290 |
| <u>Species</u> | Human |
| <u>Accession Number</u> | P62877 |
| <u>Tags</u> | N/A |
| Aminoacid sequence of the purified Rbx1 | MAAAMDVDTPSGTNSGAGKKRFEVKKWNAVALWAWDIVVDNCAICRNH IMDLCIECQANQASATSEECTVAWGVCNHFHFHCISRWLKTRQVCPL DNREWEFQKYGH |
| Native sequence | in bold |
| Protease cleavage | N/A |
| Cloning sites | Nhe1 / Kpn1 |
| DNA sequence of the Rbx1 insert | gctagc ATGGCGGCAGCGATGGATGTGGATAACCCGAGCGGCACCAAC AGCGGCGCGGGCAAGAAGCGCTTTGAAGTGAAAAAGTGGAAATGCAGTA GCCCTCTGGGCCTGGGATATTGTGGTTGATAACTGTGCCATCTGCAGG AACCACATTATGGATCTTTGCATAGAATGTCAAGCTAACCAGGCGTCC GCTACTTCAGAAGAGTGTACTGTTCGCATGGGGAGTCTGTAACCATGCT TTTCACTTCCACTGCATCTCTCGCTGGCTCAAAACACGACAGGTGTGT CCATTGGACAACAGAGAGTGGGAATTCAAAAGTATGGGCACTAG ggt acc |