

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Cullin-1 / Rbx1 / Skp1

Enzyme description:- Cullin-1 / Rbx1 / Skp1 complex (full length)

Clone number:- DU23403

Source:- Recombinant from Sf21

Tag:- cleaved from N-terminal Dac-Tev-

Purification method:- Ampicillin-Sepharose

Expression level:- 1 mg/L

Calculated molecular mass:-

Cullin-1 Monoisotopic	89621 Da
Cullin-1 Average Mass	89675 Da
Rbx1 Monoisotopic	12265 Da
Rbx1 Average Mass	12273 Da
Skp1 Monoisotopic	18051 Da
Skp1 Average Mass	18063 Da
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- Cullin-1: 8.47
Rbx1: 7.00
Skp1: 4.29

Purity:- 90 %

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 150mM NaCl, 1mM DTT

Storage temperature:- -80°C

Assay:-

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Clone Data Sheet

Protein name Cullin-1

<u>Protein</u>	Cullin-1 1-776 (full length)
<u>Synonyms</u>	Cul1
<u>Clone Number</u>	DU23403
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_003583 DNA: NM_003592
<u>Tag</u>	N-terminal Dac-TEV-
Aminoacid sequence of the expressed protein .	MSAIPGV PQIDAESYILIDYNSGKVLAEQNADVRRDPASLTKMMTSYVIG QAMKAGKFKETDLVTIGNDAWATGNPVFKGSSLMFLKPGMQVPVSQILIRG INLQSGNDACVAMADFAAGSQDAFVGLMNSYVNALGLKNTHFQTVHGLDA DGQYSSARDMALIGQALIRDVPNEYSIYKEKEFTFNIGIRQLNRNGLLWDN SLNVDGIKTGHTDKAGYNLVSATGEGQMRLISAVMGGRTFKGREAESKKL LTWGFRRFFETVNPQSENLYFQMSSTRSQNPHGLKQIGLDQIWDDLRAQIQ QVYTRQSMASRYMELYTHVYNYCTSVHQSNQARGAGVPPSKSKKGQTPG GAQFVGLELYKRLKEFLKNYLTNLLKDGEDLMDESVLKFYTQQWEDYRFS SKVLNGICAYLNRHWVRRECDEGRKGIYEIYSLALVTWRDCLFRPLNKQV TNAVCLKLIEKERNGETINTRLISGVVQSYVELGLNEDDAFAKGPTLTVYK ESFESQFLADTERFYTTRESTEFLQONPVTEYMKKAEARLLEEQRVQVYL HESTQDELARKCEQVLEKHLIEFHTEFQNLDDADKNEDLGRMYNLVSRI QDGLGELKKLLETHIHNOGLAAIEKCGEAAALNDPKMYVQTVLDVHKKYNA LVMSAFNNDAGFVAALDKACGRFINNNAVTKMAQSSSKSPELLARYCDSL LKKSSKNPEEALEDTLNQVMVVKYIEDKDVFKFYAKMLAKRLVHQNS ASDDAEASMISKLKQACGFYETSKLQRMFQDIGVSKDLNEQFKKHLTNSE PLDLDFSIOVLSSGSWPFQQSCTFALPSELESYQRFATFYASRHSGRKL TWLYQLSKGELVTNCFKNRYTLQASTFQMAILLOYNTE DAYTVQQLTDST QIKMDILAQVLQILLKSKLLVLEDENANVDEVELKPDTLIKLYLGYKNKK LRVNINVPMKTEQKQEQETHKNIEEDRKLLIQAAIVRIMKMRKVLKHQQ LLGEVLTQLSSRFKPRVPVIKKCIDILIEKEYLERVDGEKDTYSYLA
Native sequence	in bold
Protease cleavage	TEV Protease site underlined
Cloning sites	BamH1 Not1

**DNA sequence of
expression cassette**

ATGTCTGCAATCCCGGGTGTACCGCAGATCGATGCGGAGTCCTACATCCT
GATTGACTATAACTCCGGCAAAGTGCTCGCCGAACAGAACGCAGATGTCC
GCCGCGATCCTGCCAGCCTGACCAAAATGATGACCAGTTACGTTATCGGC
CAGGCAATGAAAGCCGGTAAATTTAAAGAACTGATTTAGTCACTATCGG
CAACGACGCATGGGCCACCGGTAACCCGGTGTTTAAAGGTTCTTCGCTGA
TGTTCCCTCAAACCGGGCATGCAGGTTCCGGTTTCTCAGCTGATCCGCGGT
ATTAACCTGCAATCGGGTAACGATGCTTGTGTCGCCATGGCCGATTTTGC
CGCTGGTAGCCAGGACGCTTTTGTGGCTTGATGAACAGCTACGTTAACG
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GATGGTCAGTACAGCTCCGCGCGAGATATGGCGCTGATCGGCCAGGCATT
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CAATCAAGTGATGGTTGTCTTCAAGTACATAGAAGACAAAGACGTATTTT
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GCAAGTGACGATGCCGAAGCCAGCATGATCTCCAAGTTAAAGCAAGCTTG
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AAACAGATATACTTTGCAGGCGTCGACATTCCAGATGGCTATCCTGCTTC
AGTACAACACGGAAGATGCCTACACTGTGCAGCAGCTGACCGACAGCACT
CAAATTAAAAATGGACATTTTGGCGCAAGTTTACAGATTTTATTAAAGTC
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CGGCCATCGTGAGAATCATGAAGATGAGGAAGGTTCTGAAACACCAGCAG
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CCCTGTGATCAAGAAATGCATTGACATTCTAATTGAGAAAGAATATTTGG
AGCGAGTGGATGGTGAAAAGGACACCTACAGTTACTTGGCTTAAGCGGCC
GC

Clone Data Sheet

Protein name Rbx1

<u>Protein</u>	Rbx1 1- 108 (full length)
<u>Synonyms</u>	
<u>Clone Number</u>	DU23403
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_055063 DNA: NM_014248
<u>Tag</u>	N/A-
Aminoacid sequence of the expressed protein .	MAAAMDVDTPSGTNSGAGKKRFEVKKWNAVALWAWDIVVDNCAICR NHIMDLCECQANQASATSEECTVAWGVCNHAHFHFCISRWLKTRQ VCPLDNREWEFQKYGH
Native sequence	in bold
Protease cleavage	N/A
Cloning sites	Nhe1 Kpn1
<u>DNA sequence of insert</u>	GCTAGCATGGCTGCTGCTATGGATGTTGATACCCGTCAGGCACAACTC CGGCGCTGGCAAGAAGAGATTCGAGGTGAAGAAGTGGAACGCTGTCGCTC TGTGGGCCTGGGACATCGTGGTCGATAACTGCGCTATCTGTCGTAACCAC ATCATGGACCTCTGCATCGAGTGTGAGGCCAACCAGGCTTCCGCCACCTC TGAGGAATGCACTGTTGCTTGGGGTGTGTGTAACCACGCCCTTCCACTTCC ACTGCATCAGCCGCTGGCTGAAGACCCGCCAGGTGTGCCCGCTCGACAAC AGAGAATGGGAGTTCCAGAAGTACGGTCACTAAGGTACC

Clone Data Sheet

Protein name Skp1

<u>Protein</u>	Skp1 1- 160 (full length)
<u>Synonyms</u>	EMC19, OCP2, SKP1A, TCEB1L
<u>Clone Number</u>	DU23403
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_008861 DNA: NM_006930
<u>Tag</u>	N/A-
Aminoacid sequence of the expressed protein .	MPSIKLQSSDGEIFEVDVEIAKQSVTIKTMLEDLGMDDEGDDDPVPLPNV NAAILKKVIQWCTHHKDDPPPPEDDENKEKRTDDIPVWDQEFLLKVDQGT FELILAANYLDIKGLLDVTCKTVANMIKGKTPEEIRKTFNIKNDFTEEEE AQVGSTQFCL
Native sequence	in bold
Protease cleavage	N/A
Cloning sites	PmeI AvrII multiplication module from 23370 subcloned into BstZ17I-SpeI of 23383
<u>DNA sequence of insert</u>	GGATCCATGCCTTCAATTAAGTTGCAGAGTTCTGATGGAGAGATATTTGA AGTTGATGTGGAAATTGCCAAACAATCTGTGACTATTAAGACCATGTTGG AAGATTTGGGAATGGATGATGAAGGAGATGATGACCCAGTTCCTCTACCA AATGTGAATGCAGCAATATTA AAAAAGGTCATTCAGTGGTGCACCCACCA CAAGGATGACCCTCCTCCTCCTGAAGATGATGAGAACAAAAGAAAAGCGAA CAGATGATATCCCTGTTTGGGACCAAGAATTCCTGAAAAGTTGACCAAGGA ACACTTTTTGAACTCATTCTGGCTGCAAACTACTTAGACATCAAAGGTTT GCTTGATGTTACATGCAAGACTGTTGCCAATATGATCAAGGGGAAAACTC CTGAGGAGATTCGCAAGACCTTCAATATCAAAAATGACTTTACTGAAGAG GAGGAAGCCCAGGTAGGTAGCACACAGTTTTGTCTTTGAGCGGCCGC