

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Cullin-5 / RNF7

<u>Enzyme description:-</u>	Cullin-5 / RNF7 dimer
<u>Clone number:-</u>	DU23198
<u>Source:-</u>	Recombinant
<u>Tag:-</u>	cleaved from Dac-tag
<u>Purification method:-</u>	Ampicillin Sepharose, SEC
<u>Expression level:-</u>	3mg/L

Calculated molecular mass:-

Monoisotopic	Cullin-5:	91098 Da
Average Mass	Cullin-5:	91153 Da
Monoisotopic	RNF7:	12674 Da
Average Mass	RNF7:	12683 Da

[cysteines reduced, methionines have not been oxidised]

<u>Theoretical pI:-</u>	Cullin-5: 8.54	RNF7: 5.37
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<u>Purity:-</u>	90%
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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-5 1-780 (full length)

Synonyms Cul5, VACM-1

Clone Number DU23198

Species Human

Accession Number Q93034

Tags cleaved from Dac-tag

Aminoacid sequence of
the purified and cleaved
Cullin2

**GGSMATSNLLKNKGSLQFEDKWFMRP I VLKLLRQESVTKQQWFDLFS
DVHAVCLWDDKGPAKIHQALKEDILEF IKQAQARVLSHQDDTALLKAY
IVEWRKFFTQCDILPKPFCQLEITLMGKQGSNKKSNVEDSIVRKLMLD
TWNESIFSNIKNRLQDSAMKLVHAERLGEAFDSQLVIGVRESYVNLCS
NPEDKLOIYRDNFEKAYLDSTERFYRTQAPSYLOQNGVQNYMKYADAK
LKEEEKRALRYLETRRECNSVEALMECCVNALVTSFKETILAECQGM
KRNETEKLHLMFSLMDKVPNGIEPMLKDLEHIISAGLADMVAAAETI
TTDSEKYVEQLLTLFNRF SKLVKEAFQDDPRFLTARDKAYKAVVNDAT
IFKLELPLKQKGVGLKTQPE SKCPELLANYCDMLLRKTPLSKKL TSEE
IEAKLKEVLLVLKYVQNKDVFMRYPHKAHLTRRLILDISADSEIEENMV
EWLREVGMPADYVNKLARMFQDIKVSEDLNQAFKEMHKNNKLALPADS
VNIKILNAGAWSRSSEKVFVSLPTELEDLIPVEEFYKKNHSGRKLHW
HHLMSGIITFKNEVGQYDLEVTTFQLAVLFAWNQRPREKISFENLKL
ATELPDAELRRTLWLSLVAFPKLKRQVLLYEPQVNSPKDFTEGTLFSVN
QEFSLIKNAKQKRGKINLIGRLQLTTERMREEENEGIVQLRILRTQE
AIIQIMKMRKKISNAQLQTELVEILKNMFLPQKKMIKEQIEWLIEHKY
IRRDESDINTFIYMA**

Native sequence in bold

Protease cleavage TEV site underlined

Cloning sites BamH1 / NotI

**DNA sequence of
the Cullin-5 insert**

ggatccATGGCGACGTCTAATCTGTAAAGAATAAAGGTTCTCTTCAGTTTG
AAGACAAATGGGATTTTATGCGCCCGATTGTTTTGAAGCTTTTACGCCAGGA
ATCTGTTACAAAACAGCAGTGGTTGATCTGTTTTCGGATGTGCATGCAGTC
TGTCTTTGGGATGATAAAGGCCAGCAAAAATTCATCAGGCTTTAAAAGAAG
ATATTCTTGAGTTTATTAAGCAAGCACAGGCACGAGTACTGAGCCATCAAGA
TGATACGGCTTTGCTAAAAGCATATATTGTTGAATGGCGAAAAGTTCTTTACA
CAATGTGATATTTTACCAAACCTTTTTGTCAACTAGAGATTACTTTAATGG
GTAAACAGGGCAGCAATAAAAAATCAAATGTGGAAGACAGTATTGTTTCGAAA
GCTTATGCTTGATACATGGAATGAGTCAATCTTTTCAAACATAAAAAACAGA
CTCCAAGATAGTGCAATGAAGCTGGTACATGCTGAGAGATTGGGAGAAGCTT
TTGATTCTCAGCTGGTTATTGGAGTAAGAGAATCCTATGTTAACCTTTGTTT
TAATCCTGAGGATAAACCTCAAATTTATAGGGACAATTTTGAGAAGGCATAC
TTGGATTCAACAGAGAGATTTTATAGAACAAGCACCCTCGTATTTACAAC
AAAATGGTGTACAGAATTATATGAAATATGCAGATGCTAAATTTAAAAGAAGA
AGAAAAACGAGCACTACGTTATTTAGAAACAAGACGAGAATGTAACTCCGTT
GAAGCACTCATGGAATGCTGTGTAATGCCCTGGTGACATCATTTAAAGAGA
CTATCTTAGCTGAGTGCCAAGGCATGATCAAGAGAAATGAACTGAAAAATT
ACATTTAATGTTTTCATTTGATGGACAAAGTTCCTAATGGTATAGAGCCAATG
TTGAAAGACTTGGAGGAACATATCATTAGTGCTGGCCTGGCAGATATGGTAG
CAGCTGCTGAAACTATTACTACTGACTCTGAGAAATACGTTGAGCAGTTACT
TACACTATTTAATAGATTTAGTAACTCGTCAAAGAAGCTTTTCAAGATGAT
CCACGATTTCTTACTGCAAGAGATAAGGCGTATAAAGCAGTTGTTAATGATG
CTACCATATTTAACTTGAATTACCTTTGAAGCAGAAGGGGGTGGGATTTAAA
AACTCAGCCTGAATCAAATGCCCTGAGCTGCTTGCCAATTACTGTGACATG
TTGCTAAGAAAAACACCATTAAGCAAAAAACTAACCTCTGAAGAGATTGAAG
CAAAGCTTAAAGAAGTGCTCTTGGTACTTAAGTATGTACAGAACAAGATGT
TTTTATGAGGTATCATAAAGCTCATTTGACACGACGTCTTATATTAGACATC
TCTGCCGATAGTGAAATTGAAGAAAACATGGTAGAGTGGCTAAGAGAAGTTG
GTATGCCAGCGGATTATGTAAACAAGCTTGCTAGAATGTTTCAGGACATAAAA
AGTATCTGAAGATTTGAACCAAGCTTTTAAAGGAAATGCACAAAAATAATAAA
TTGGCATTACCAGCTGATTCAGTTAATATAAAAAATCTGAATGCTGGCGCCT
GGTCAAGAAGTTCTGAGAAAGTCTTTGTCTCACTTCCTACTGAACTGGAGGA
CTTGATACCGGAAGTAGAAGAATTCTACAAAAAAAATCATAGTGGTAGAAAA
TTACATTGGCATCATCTCATGTCAAATGGAATTATAACATTTAAGAATGAAG
TTGGTCAATATGATTTGGAGGTAACCACGTTTCAGCTCGCTGTATTGTTTGC
ATGGAACCAAAGACCCAGAGAGAAAATCAGCTTTGAAAATCTTAAGCTTGCA
ACTGAACTCCCTGATGCTGAACTTAGGAGGACTTTATGGTCTTTAGTAGCTT
TCCCAAACTCAAACGGCAAGTTTTGTTGTATGAACCTCAAGTCAACTCACC
CAAAGACTTTACAGAAGGTACCCTCTTCTCAGTGAACCAGGAGTTTCAGTTTA
ATAAAAAATGCAAAGTTTCAGAAAAGGGGTAAAAATCAACTTGATTGGACGTT
TGCAGCTCACTACAGAAAGGATGAGAGAAGAAGAGAATGAAGGAATAGTTCA
ACTACGAATACTAAGAACCAGGAAGCTATCATACAAATAATGAAAATGAGA
AAGAAAATTAGTAATGCTCAGCTGCAGACTGAATTAGTAGAAATTTTGAAAA
ACATGTTCTTGCCACAAAAGAAAATGATAAAAGAGCAAATAGAGTGGCTAAT
AGAGCACAAATACATCAGAAGAGATGAATCTGATATCAACACTTTCATATAT
ATGGCATAAGCGGCCGC

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Clone Data Sheet

Protein name

<u>Protein</u>	RNF7 1-113 (full length)
<u>Synonyms</u>	Rbx2, SOC2, SAG
<u>Clone Number</u>	DU23198
<u>Species</u>	Human
<u>Accession Number</u>	Q9UBF6
<u>Tags</u>	N/A
Aminoacid sequence of the purified Rbx1	MADVEDGEETCALASHSGSSGSKSGGDKMFLKKWNAVAMWSWDVECD TCAICRVQVMDACLRCQAENKQEDCVVVWGECNHSFHNCCMSLWVKQN NRCPLCQQDWVVRIGK
Native sequence	in bold
Protease cleavage	N/A
Cloning sites	Nhe1 / Kpn1
DNA sequence of the Rbx1 insert	ATGGCCGACGTGGAAGACGGAGAGGAAACCTGCGCCCTGGCCTCTCAC TCCGGGAGCTCAGGCTCCAAGTCGGGAGGCGACAAGATGTTCTCCCTC AAGAAGTGGAACGCGGTCCGATGTGGAGCTGGGACGTGGAGTGCAT ACGTGCGCCATCTGCAGGTCCAGGTGATGGATGCCTGTCTTAGATGT CAAGCTGAAAACAAACAAGAGGACTGTGTTGTGGTCTGGGGAGAAATGT AATCATTCCTTCCACAACCTGCTGCATGTCCCTGTGGGTGAAACAGAAC AATCGCTGCCCTCTCTGCCAGCAGGACTGGGTGGTCCAAAGAATCGGC AAATGA