

CBL [GST-tagged]

E3 Ligase

Alternate Names: Casitas B-lineage lymphoma proto-oncogene, Cas-Br-M ectropic retroviral transforming sequence, RING finger protein 55, proto-oncogene c-CBL, CBL2, and RNF55

Cat. No. 63-0004-010

Lot. No. 1422

Quantity: 10 µg

Storage: -70°C



FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). Cas-Br-M Murine Ecotropic Retroviral Transforming Sequence Homolog (CBL) is a member of the E3 protein ligase family and cloning of the human gene was first described by Langdon *et al.* (1989). A single c-CBL locus termed CBL2 has been mapped to human chromosome 11q23. This region of chromosome 11 is involved in translocations and deletions in a broad range of leukaemias; c-CBL has been found to be translocated from chromosome 11 in leukaemias with either t(4;11) or t(11;14) abnormalities (Savage *et al.*, 1991; Wei *et al.*, 1990). Ubiquitylation of receptor protein-tyrosine kinases (rPTKs) terminates signalling by marking active receptors for degradation. CBL is an adaptor protein for rPTKs. Recent studies have shown that CBL is a positive regulator of rPTK ubiquitylation by activating E2 enzymes through its RING finger domain and targeting substrates such as the Platelet Derived Growth Factor Receptor (PDGFR) through its SH2 domain (Joazeiro *et al.*, 1999). Ubiquitylation of the Hepatocyte Growth Factor (HGF) receptor by CBL has been shown to recruit the endophilin-CIN85 complex resulting in receptor internalisation and

Continued on page 2

Physical Characteristics

Species: human

Source: *E. coli* expression

Quantity: 10 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~126 kDa

Purity: >50% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70°C; aliquot as required

Protein Sequence: Please see page 2

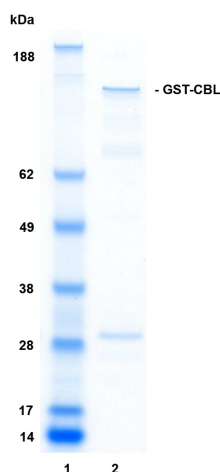
Quality Assurance

Protein Identification:

Confirmed by mass spectrometry.

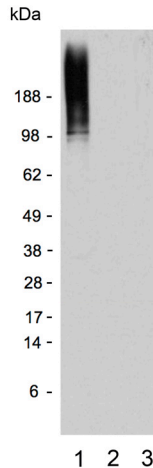
Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-CBL



E3 ligase assay:

The ubiquitin conjugating activity of GST-CBL was validated through its ability to catalyse the generation of polyubiquitin chains in the presence of the E1 activating enzyme His-UBE1, the E2 conjugating enzyme His-UBE2D2 (UbcH5b) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of GST-CBL for 30 minutes at 30°C in the presence of ubiquitin, His-UBE1, His-UBE2D2 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-CBL (Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an anti-ubiquitin conjugate antibody and these were observed only in the presence of both ATP and GST-CBL.



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Lot-specific COA version tracker: v1.0.1

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Background

Continued from page 1

degradation (Petrelli, Gilestro *et al.* 2002). Mutations in a highly conserved alpha helical structure of CBL linking the SH2 and RING finger domains renders CBL proteins oncogenic (Thien *et al.*, 2001). C-CBL(-/-) haematopoietic stem/progenitor cells (HSPCs) have shown enhanced sensitivity to a variety of cytokines compared to C-CBL(+/-) HSPCs. Furthermore, homozygous C-CBL mutations have been found in most 11q-aUPD-positive myeloid malignancies (Sanada *et al.*, 2009).

References:

Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin LY, PatJoazeiro CA, Wing SS, Huang H, Levenson JD, Hunter T, Liu YC (1999) The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. *Science* **286**, 309-12.

Langdon WY, Hartley JW, Klinken SP, Ruscetti SK, Morse HC, 3rd (1989) v-cbl, an oncogene from a dual-recombinant murine retrovirus that induces early B-lineage lymphomas. *Proc Natl Acad Sci U S A* **86**, 1168-72.

Petrelli A, Gilestro GF, Lanzardo S, Comoglio PM, Migone N, Giordano S (2002) The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature* **416**, 187-90.

Sanada M, Suzuki T, *et al.* (2009) Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. *Nature* **460**, 904-8.

Savage PD, Shapiro M, Langdon WY, Geurts van Kessel AD, Seuanez HN, Akao Y, Croce C, Morse HC, 3rd, Kersey JH (1991) Relationship of the human protooncogene CBL2 on 11q23 to the t(4;11), t(11;22), and t(11;14) breakpoints. *Cytogenet Cell Genet* **56**, 112-5.

Thien CB, Walker F, Langdon WY (2001) RING finger mutations that abolish c-Cbl-directed polyubiquitination and downregulation of the EGF receptor are insufficient for cell transformation. *Mol Cell* **7**, 355-65.

Wei S, Rocchi M, Archidiacono N, Sacchi N, Romeo G, Gatti RA (1990) Physical mapping of the human chromosome 11q23 region containing the ataxia-telangiectasia locus. *Cancer Genet Cytogenet* **46**, 1-8.

Physical Characteristics

Continued from page 1

Protein Sequence:

**MSPILGYWKIKGLVQPTRLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNM
LGGCPKERA EISMLEGAVLDIRYGVS
RIAYS KDFETLKVDFLSKLP EMLKMF E
DRLCHKTYLNGDHVTHPDFMLYDALDV
VLYMDPMLCLDAFPKLVCFKKRIEAIPO
IDKYLKSSKYIAWPLQGWQATFGGGDHP
PKSD **LEVL**FQGPLGSMAGNVKKSSGAG
GGSGSGSGSGGLIGLMKDAFQPHHHH
HHLSPHPPGTVDKKMVEKCKWKLMDKVVR
LCQNPKLALKNSPPIILDLLPDTYQHL
RTLILSRYEGKMETLGENEYFRVFMENLMK
KTKQITISL FKEGKERMYEENSQPRRNL T
KLSLIFSHMLAELKGIFFSGLFQGDTRIT
KADAAEFWRKAFGEKTI VPKSFRQAL
HEVHPISSGLEAMALKSTIDLTCNDYIS
VFEFDIFTRLFQPWSSLLRNWNSLAVTH
PGYMAFLTYDEVKARLQKF I HKPGSY I
FRLSCTR LGQWAIGYVTADGNILQTI PHN
KPLFQALIDGFRREGFYLPDGRNQNPDLT
GLCEPTQDHKVTQEYELYCEMGSTFQL
CKICAENDKDKVIEPCGHLMCTSLTSWQE
SEGQGCPCRC EIKGTEPIVVDPFDRGSG
SLLRQGAEGAPSPNYDDDDDERADDTLFM
MKELAGAKVERPPSPFSMAPQASLPPVP
PRLDLLPQRVCVPSASALGTASKAASG
SLHKDKPLPVPPTLRDLPPPPPPDRPYS
VGAESRPQRRPLPCTPGDCPSRDKLP
PVPSSRLGDSWLPRPIPKVPVSA P S S D P
WTGRELTNRHSLPFSLP SQMEPRPDVPRLG
STFSLDTSMSMNSSPLVGPECDHPKIKPSS
SANAIYSLAARPLPVPKLP PGEQCEGE
EDTEYMT P S SRPLRPLDTSQSSRACDCDQ Q
ID SCTYEAM YNIQS QAPSITESSTFGEGN
LAAAHANTGPEESENEDDGYDVPKPPVPAV
LARRTLSDISNASSSFGWLSLDGDPTT
NVTEGSQVPERPPKPFRRINSEKAGSC
QQGSGPAASAAATASPQLSSEIENLMSQ
GYSYQDIQKALVIAQNNIEMAKNILREFV
SISSPAHVAT**

Tag (**bold text**): N-terminal GST

Protease cleavage site: PreScission™ (**LEVL**FQ▼**GP**)

CBL (regular text): Start **bold italics** (amino acid residues 1-906)

Accession number: NP_005179.2



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