

Anti-CBL Antibody Picoband™

Catalog Number: PB9077

About CBL

CBL (Cbl proto-oncogene) is also known as C-CBL, RNF55, CBL2 and E3 ubiquitin protein ligase. CBL is mapped to chromosome 11q23.3-qter by molecular characterization of the breakpoints in 2 somatic cell hybrids. The encoded protein is one of the enzymes required for targeting substrates for degradation by the proteasome. This protein mediates the transfer of ubiquitin from ubiquitin conjugating enzymes (E2) to specific substrates. This protein also contains an N-terminal phosphotyrosine binding domain that allows it to interact with numerous tyrosine-phosphorylated substrates and target them for proteasome degradation. As such it functions as a negative regulator of many signal transduction pathways. This gene has been found to be mutated or translocated in many cancers including acute myeloid leukaemia. Mutations in this gene are also the cause of Noonan syndrome-like disorder.

Overview

Product Name	Anti-CBL Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-CBL Antibody Picoband™ catalog # PB9077. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P22681

Technical Details

Immunogen	E.coli-derived human CBL recombinant protein (Position: A556-T906). Human CBL shares 84% amino acid (aa) sequence identity with mouse CBL.
Predicted Reactive Species	Bovine, Canine, Monkey, Rabbit
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG





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Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat, By Heat Immunocytochemistry/Immunofluorescence, 2ug/ml, Human Flow Cytometry, 1-3ug/1x10 ⁶ cells, Human



Anti-CBL Antibody Picoband™ (PB9077) Images

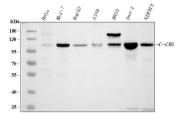


Figure 1. Western blot analysis of CBL using anti-CBL antibody (PB9077).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,

Lane 2: human MCF-7 whole cell lysates,

Lane 3: human HepG2 whole cell lysates,

Lane 4: human A549 whole cell lysates,

Lane 5: rat RH35 whole cell lysates,

Lane 6: mouse Ana-1 whole cell lysates,

Lane 7: mouse NIH/3T3 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-CBL antigen affinity purified polyclonal antibody (Catalog # PB9077) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for CBL at approximately 120 kDa. The expected band size for CBL is at 100,120 kDa.

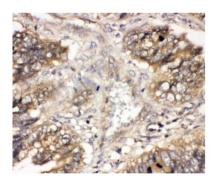


Figure 2. IHC analysis of CBL using anti-CBL antibody (PB9077).

CBL was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-CBL Antibody (PB9077) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

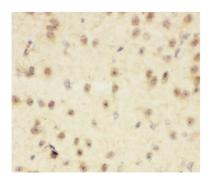


Figure 3. IHC analysis of CBL using anti-CBL antibody (PB9077).

CBL was detected in paraffin-embedded section of mouse brain tissues. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-CBL Antibody (PB9077) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



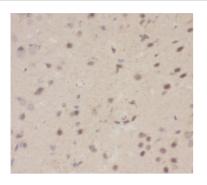


Figure 4. IHC analysis of CBL using anti-CBL antibody (PB9077).

CBL was detected in paraffin-embedded section of rat brain tissues. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-CBL Antibody (PB9077) overnight at 4°C. Biotinylated goat antirabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

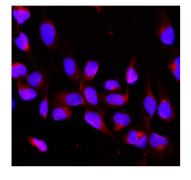


Figure 5. IF analysis of CBL using anti-CBL antibody (PB9077).

CBL was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-CBL Antibody (PB9077) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG (BA1142) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

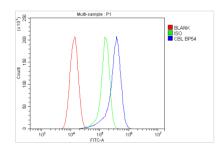


Figure 6. Flow Cytometry analysis of A549 cells using anti-CBL antibody (PB9077).

Overlay histogram showing A549 cells stained with PB9077 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-CBL Antibody (PB9077, $1ug/1x10^6$ cells) for 30 min at 20° C. DyLight® 488 conjugated goat anti-rabbit IgG (BA1127, 5- $10ug/1x10^6$ cells) was used as secondary antibody for 30 minutes at 20° C. Isotype control antibody (Green line) was rabbit IgG ($1ug/1x10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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