

ORDERING INFORMATION

Catalog Number: AF5998

Lot Number: CDLF01

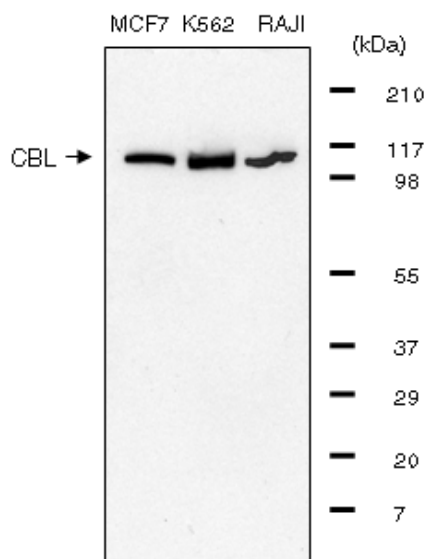
Size: 100 µg

Specificity: human CBL

Immunogen: *E. coli*-derived rhCBL
(aa 775 - 906)

Ig Type: goat IgG

Application: Western blot



Detection of CBL with AF5998.

30 µg of whole cell extracts from MCF7, K562, and RAJI cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 0.5 µg/mL goat anti-CBL antibody, as described in *Protocols for Immunoblotting*.

Background

CBL (Lake Casitas, California mouse B-lineage Lymphoma; also RING finger protein 55) is a 120 - 125 kDa cytosolic member of the CBL family of proteins. It is widely expressed, being found in cells such as fibroblasts, adipocytes, osteoclasts, podocytes and mammary epithelium. CBL negatively regulates RTK signaling by acting as an E3 ubiquitin ligase. Following activation via phosphorylation, CBL will interact with multiple signaling molecules such as Src, ZAP-70, EGFR and SHP1 terminating their activation. Human CBL is 906 amino acids (aa) in length. It contains an N-terminal phosphoTyr-binding region with one α -helix bundle, an EF hand segment, and an SH2 domain (aa 47 - 342), a RING finger domain that interacts with E2 enzymes (aa 381 - 423) a Pro-rich SH3 domain (aa 477 - 688), and a Leu-zipper/Ubiquitin-associated region (aa 861 - 892). There is one potential alternative start site at Met55. Over aa 775 - 906, human CBL shares 91% aa identity with mouse CBL.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human CBL (rhCBL; aa 775 - 906; Accession # P22681). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% Na₂S₂O₃.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects endogenous human CBL in Western blot with an approximate molecular weight of 120 kDa.

Application

Western blot - An antibody concentration of 0.5 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane 1 hour at room temperature in Antibody Solution containing 0.5 µg/mL goat anti-human CBL.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:2000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - A single plate (150 mm) of exponentially growing cells is washed twice in cold PBS. 1 mL of boiling 1% SDS lysis buffer (1% SDS, 10 mM Tris-HCl, pH 7.4, 1 mM sodium ortho-vanadate) is added to the plate. The plate is then scraped and the lysis is collected, sonicated and quantified. 30 µg of cellular protein is added to an equal amount of 2x SDS loading buffer. Samples are then boiled for 5 minutes and run on a SDS-PAGE gel.

Optimal dilutions should be determined by each laboratory for each application.