abcam

Product datasheet

Anti-CBLB antibody [246C5a] ab54362

KO VALIDATED

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Overview

Product name	Anti-CBLB antibody [246C5a]
Description	Mouse monoclonal [246C5a] to CBLB
Host species	Mouse
Tested applications	**Suitable for:** IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant C terminal fragment of Human CBLB.
Positive control	WB: CTLL-2, HeLa and MCF7 cell lysates
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term.
Storage buffer	pH: 7.40 Preservative: 0.05% Sodium azide Constituents: 1% BSA, PBS
Purity	Protein G purified
Purification notes	ab54362 was purified using protein G column chromatography from culture supernatant of hybridoma cultured in a medium containing bovine IgG depleted (approximately 95%) fetal bovine serum and filtered through a 0.22µm membrane.
Clonality	Monoclonal
Clone number	246C5a

Isotype IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab54362 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

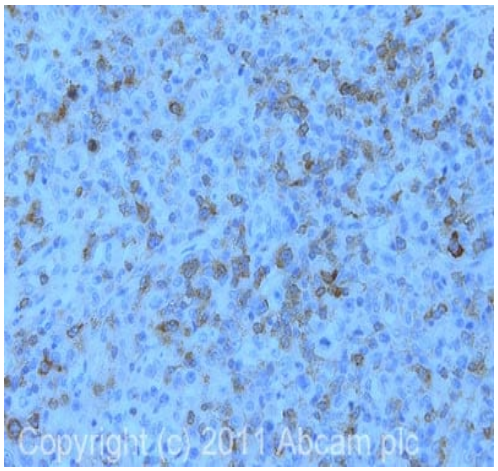
Application	Abreviews	Notes
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	★★★★☆ (1)	Use at an assay dependent concentration. Predicted molecular weight: 109 kDa.

Target

Relevance Function: E3 ubiquitin-protein ligase which accepts ubiquitin from specific E2 ubiquitin-conjugating enzymes, and transfers it to substrates, generally promoting their degradation by the proteasome. Negatively regulates TCR (T-cell receptor), BCR (B-cell receptor) and FCER1 (high affinity immunoglobulin epsilon receptor) signal transduction pathways. In naive T-cells, inhibits VAV1 activation upon TCR engagement and imposes a requirement for CD28 costimulation for proliferation and IL-2 production. Also acts by promoting PIK3R1/p85 ubiquitination, which impairs its recruitment to the TCR and subsequent activation. In activated T-cells, inhibits PLCG1 activation and calcium mobilization upon restimulation and promotes anergy. In B-cells, acts by ubiquitinating SYK and promoting its proteasomal degradation. Slightly promotes SRC ubiquitination. May be involved in EGFR ubiquitination and internalization. May be functionally coupled with the E2 ubiquitin-protein ligase UB2D3. Tissue specificity: Expressed in placenta, heart, lung, kidney, spleen, ovary and testis, as well as fetal brain and liver and hematopoietic cell lines, but not in adult brain, liver, pancreas, salivary gland, or skeletal muscle. Present in lymphocytes (at protein level). Pathway: Protein modification; protein ubiquitination. Similarity: Contains 1 Cbl-PTB (Cbl-type phosphotyrosine-binding) domain. Contains 1 RING-type zinc finger. Contains 1 UBA domain. Domain: The N-terminus is composed of the phosphotyrosine binding (PTB) domain, a short linker region and the RING-type zinc finger. The PTB domain, which is also called TKB (tyrosine kinase binding) domain, is composed of three different subdomains: a four-helix bundle (4H), a calcium-binding EF hand and a divergent SH2 domain. The RING-type zinc finger domain mediates binding to an E2 ubiquitin-conjugating enzyme. The UBA domain interacts with poly-ubiquitinated proteins. PTM: Phosphorylated on tyrosine and serine residues upon TCR or BCR activation, and upon various types of cell stimulation. Auto-ubiquitinated upon EGF-mediated cell activation or upon T-cell costimulation by CD28; which promotes proteasomal degradation.

Cellular localization Cytoplasmic and Nuclear

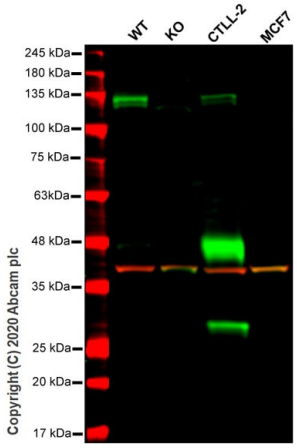
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CBLB antibody [246C5a] (ab54362)

IHC image of ab54362 staining in human T-cell lymphoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab54362, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-CBLB antibody [246C5a] (ab54362)

All lanes : Anti-CBLB antibody [246C5a] (ab54362) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CBLB knockout HeLa cell lysate
Lane 3 : CTLL-2 cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

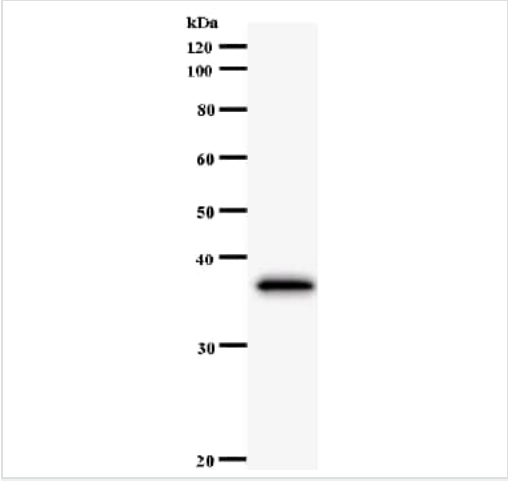
Performed under reducing conditions.

Predicted band size: 109 kDa
Observed band size: 130 kDa

Lanes 1-4: Merged signal (red and green). Green - ab54362 observed at 130 kDa. Red - loading control, **ab181602** observed at 37 kDa.

ab54362 Anti-CBLB antibody [246C5a] was shown to specifically react with CBLB in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab264711** (knockout cell lysate **ab258342**) was used. Wild-type and CBLB knockout samples were subjected to SDS-PAGE. ab54362 and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab181602**) were incubated

overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-CBLB antibody [246C5a] (ab54362)

Anti-CBLB antibody [246C5a] (ab54362) + immunizing recombinant protein

Predicted band size: 109 kDa
Observed band size: 37 kDa

The molecular weight of the band on the Western blot does not correspond to the molecular weight of the natural protein as the immunizing recombinant protein fragment was used as the test antigen.

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