abcam

Product datasheet

Anti-CBL antibody [YE323] - BSA and Azide free ab236075

KO VALIDATED Recombinant RabMAb

7 Images

Overview

Product name	Anti-CBL antibody [YE323] - BSA and Azide free
Description	Rabbit monoclonal [YE323] to CBL - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Chicken
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Epitope	ab236075 reacts with an epitope located in the C terminal region of CBL.
Positive control	WB: HEK293T, HAP1, Jurkat, THP-1, WEHI-231, F9 and Raji cell lysates; Mouse thymus tissue lysate, Rat testis lysate, Rat thymus lysate. ICC/IF: Jurkat cells. Flow Cyt (intra): Jurkat cells.
General notes	ab236075 is the carrier-free version of **ab32027**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production

For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	YE323
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab236075 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
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 120 kDa (predicted molecular weight: 99 kDa).
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

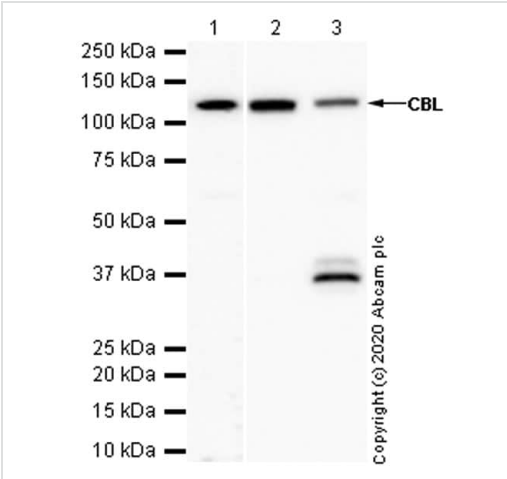
Function Participates in signal transduction in hematopoietic cells. Adapter protein that functions as a negative regulator of many signaling pathways that start from receptors at the cell surface. Acts as an E3 ubiquitin-protein ligase, which accepts ubiquitin from specific E2 ubiquitin-conjugating enzymes, and then transfers it to substrates promoting their degradation by the proteasome. Recognizes activated receptor tyrosine kinases, including PDGFA, EGF and CSF1, and terminates signaling.

Pathway Protein modification; protein ubiquitination.

Involvement in disease Defects in CBL are the cause of Noonan syndrome-like disorder (NSL) [MIM:613563]. NSL is a syndrome characterized by a phenotype reminiscent of Noonan syndrome. Clinical features are

	highly variable, including facial dysmorphism, short neck, developmental delay, hyperextensible joints and thorax abnormalities with widely spaced nipples. The facial features consist of triangular face with hypertelorism, large low-set ears, ptosis, and flat nasal bridge. Some patients manifest cardiac defects.
Sequence similarities	Contains 1 Cbl-PTB (Cbl-type phosphotyrosine-binding) domain. Contains 1 RING-type zinc finger. Contains 1 UBA domain.
Domain	The RING-type zinc finger domain mediates binding to an E2 ubiquitin-conjugating enzyme. 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.
Post-translational modifications	Phosphorylated on tyrosine residues by EGFR, SYK, FYN and ZAP70 (By similarity). Phosphorylated on tyrosine residues by INSR.
Cellular localization	Cytoplasm.

Images



Western blot - Anti-CBL antibody [YE323] - BSA and Azide free (ab236075)

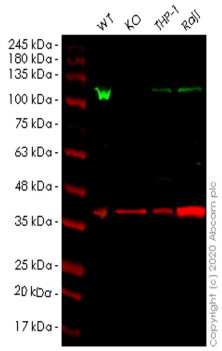
All lanes : Anti-CBL antibody [YE323] - C-terminal (**ab32027**) at 1/1000 dilution

Lane 1 : Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate at 15 µg
Lane 2 : Rat testis lysate at 20 µg
Lane 3 : Rat thymus lysate at 20 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/2000 dilution

Predicted band size: 99 kDa
Observed band size: 110 kDa

This data was developed using **ab32027**, the same antibody clone in a different buffer formulation.



Western blot - Anti-CBL antibody [YE323] - BSA and Azide free (ab236075)

All lanes : Anti-CBL antibody [YE323] - C-terminal (**ab32027**) at 1/1000 dilution (unpurified)

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : CBL knockout HEK293T cell lysate
Lane 3 : THP-1 cell lysate
Lane 4 : Raji cell lysate

Lysates/proteins at 20 µg per lane.

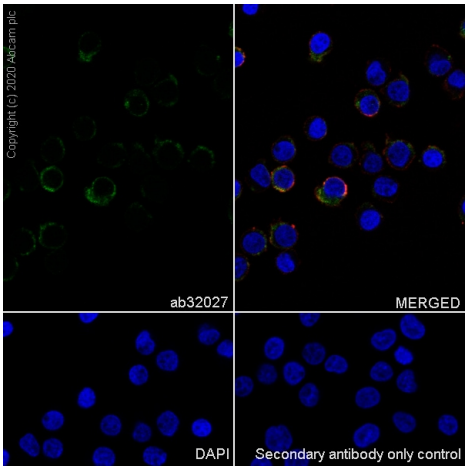
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 99 kDa
Observed band size: 110 kDa

This data was developed using **ab32027**, the same antibody clone in a different buffer formulation.

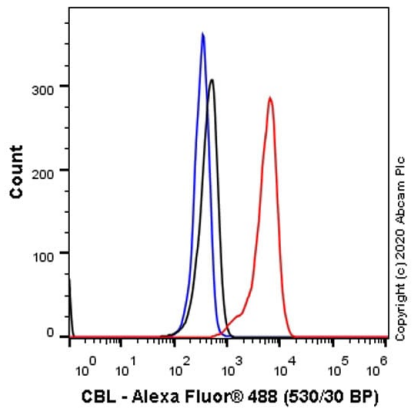
Lanes 1-4: Merged signal (red and green). Green - **ab32027** observed at 110 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab32027 Anti-CBL antibody [YE323] - C-terminal was shown to specifically react with CBL in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab267245** (knockout cell lysate **ab257200**) was used. Wild-type and CBL knockout samples were subjected to SDS-PAGE. **ab32027** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



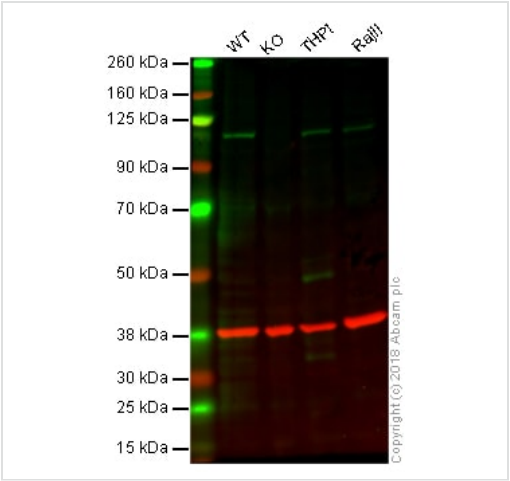
Immunocytochemistry/ Immunofluorescence - Anti-CBL antibody [YE323] - BSA and Azide free (ab236075)

This data was developed using **ab32027**, the same antibody clone in a different buffer formulation.
Immunocytochemistry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling CBL with purified **ab32027** at 1/50 dilution (4.26 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-CBL antibody [YE323] - BSA and Azide free (ab236075)

This data was developed using **ab32027**, the same antibody clone in a different buffer formulation. Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling CBL with purified **ab32027** at 1/30 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



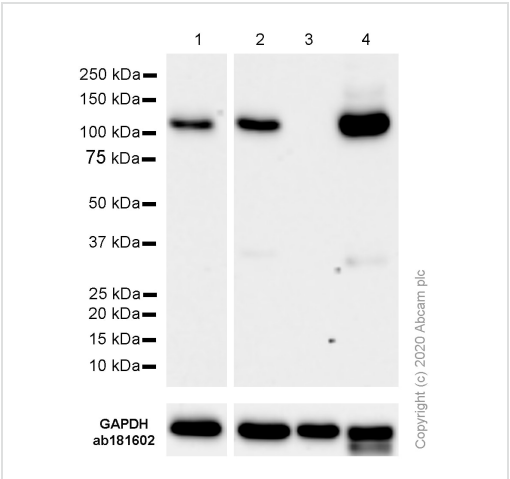
Western blot - Anti-CBL antibody [YE323] - BSA and Azide free (ab236075)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: CBL knockout HAP1 whole cell lysate (20 µg)
Lane 3: THP1 whole cell lysate (20 µg)
Lane 4: Raji whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - **ab32027** observed at 100 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

Unpurified **ab32027** was shown to specifically react with CBL in wild-type HAP1 cells as signal was lost in CBL knockout cells. Wild-type and CBL knockout samples were subjected to SDS-PAGE. **ab32027** and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32027**).



Western blot - Anti-CBL antibody [YE323] - BSA and Azide free (ab236075)

All lanes : Anti-CBL antibody [YE323] - C-terminal (**ab32027**) at 1/1000 dilution

Lane 1 : WEHI-231 (Mouse B cell lymphoma B lymphocyte) cell lysate
Lane 2 : F9 (Mouse embryonal carcinoma epithelial cell) cell lysate
Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) cell lysate
Lane 4 : Mouse thymus lysate

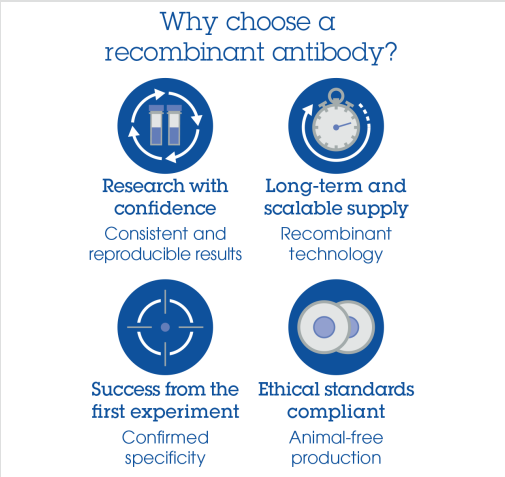
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 99 kDa
Observed band size: 110 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 30 seconds



Anti-CBL antibody [YE323] - BSA and Azide free

(ab236075)

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