


Product datasheet

Anti-CBL antibody [YE323] - C-terminal ab32027

KO VALIDATED Recombinant RabMAB

[11 References](#) [7 Images](#)

Overview

Product name	Anti-CBL antibody [YE323] - C-terminal
Description	Rabbit monoclonal [YE323] to CBL - C-terminal
Host species	Rabbit
Tested applications	Suitable for: WB, Flow Cyt (Intra), ICC/IF Unsuitable for: IP
Species reactivity	Reacts with: Mouse, Rat, Human Predicted to work with: Chicken 
Immunogen	Synthetic peptide within Human CBL aa 850 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: P22681
Epitope	ab32027 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	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p> <p>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</p>

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: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	YE323
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32027 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
WB		1/1000. Detects a band of approximately 120 kDa (predicted molecular weight: 99 kDa). For unpurified use at 1/5000
Flow Cyt (Intra)		1/30. For unpurified use at 1/100 ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50. For unpurified use at 1/100

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.

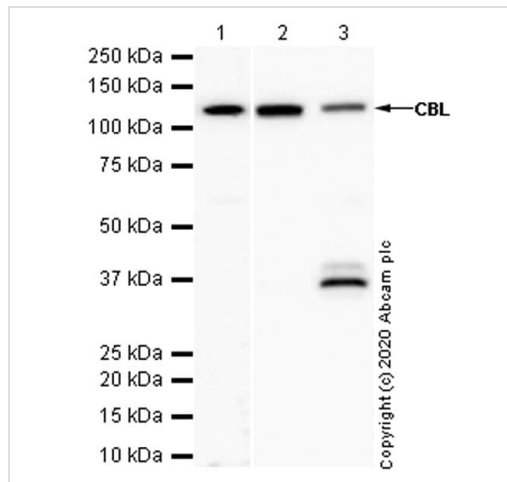
Post-translational modifications

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] - C-terminal (ab32027)

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

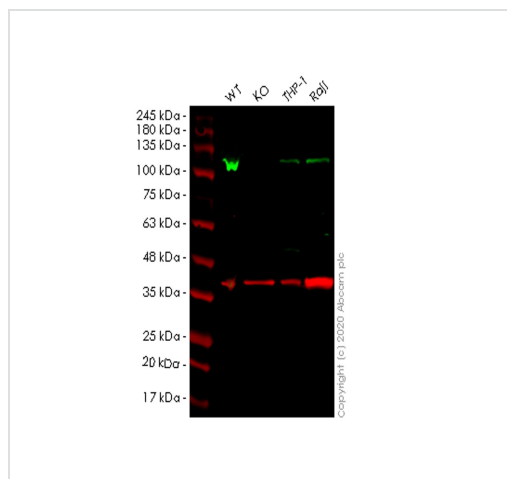
Lane 3 : Rat thymus lysate at 20 µg

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



Western blot - Anti-CBL antibody [YE323] - C-terminal (ab32027)

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

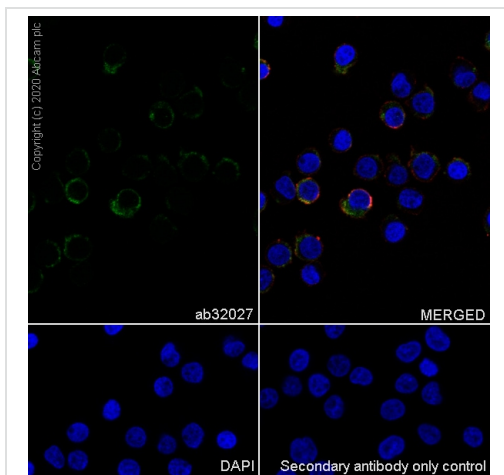
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution

Predicted band size: 99 kDa

Observed band size: 110 kDa

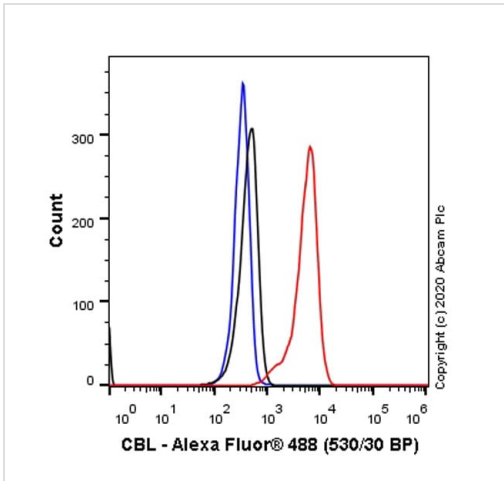
Lanes 1-4: Merged signal (red and green). Green - ab32027 observed at 110 kDa. Red - loading control **ab8245** observed at 36 kDa.

Unpurified 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.



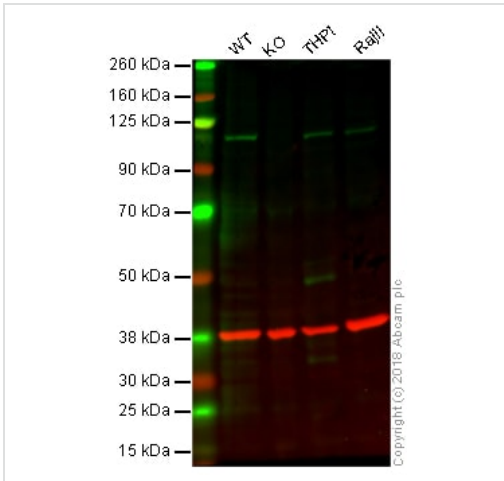
Immunocytochemistry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling CBL with purified ab32027 at 1/50 dilution (4.26 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - 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.

Immunocytochemistry/ Immunofluorescence - Anti-CBL antibody [YE323] - C-terminal (ab32027)



Flow Cytometry (Intracellular) - Anti-CBL antibody [YE323] - C-terminal (ab32027)

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] - C-terminal (ab32027)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

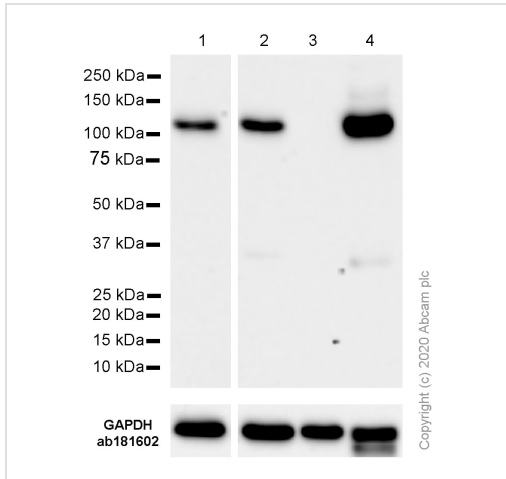
Lane 2: CBL knockout HAP1 whole cell lysate (20 µg)

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.



Western blot - Anti-CBL antibody [YE323] - C-terminal (ab32027)

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% NFD/MTBST

Exposure time: 30 seconds

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-CBL antibody [YE323] - C-terminal (ab32027)

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