abcam

Product datasheet

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





11 References 7 Images

Overview

Product name Anti-CBL antibody [YE323] - C-terminal

Rabbit monoclonal [YE323] to CBL - C-terminal **Description**

Host species Rabbit

Tested applications Suitable for: WB, Flow Cyt (Intra), ICC/IF

Unsuitable for: IP

Reacts with: Mouse, Rat, Human Species reactivity

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

ab32027 reacts with an epitope located in the C terminal region of CBL. **Epitope**

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 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: 7.20

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 <u>ab172730</u> - Rabbit monoclonal lgG, 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.

T	a	rg	g	et

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.

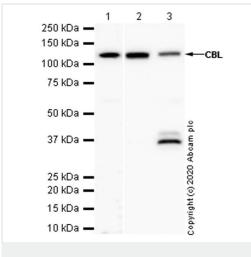
Domain

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

Phosphorylated on tyrosine residues by EGFR, SYK, FYN and ZAP70 (By similarity).

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

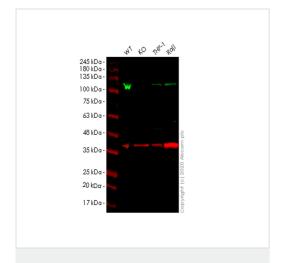
Lane 2 : Rat testis lysate at 20 μg Lane 3 : Rat thymus lysate at 20 μg

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 lgG 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 <u>ab8245</u> observed at 36 kDa.

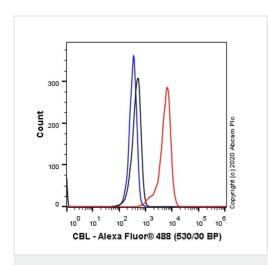
Unpurified ab32027 Anti-CBL antibody [YE323] - C-terminal was shown to specifically react with CBL in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab267245 (knockout cell lysate ab257200) was used. Wild-type and CBL knockout samples were subjected to SDS-PAGE. ab32027 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 lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

ab32027 MERGED

DAPI Secondary antibody only control

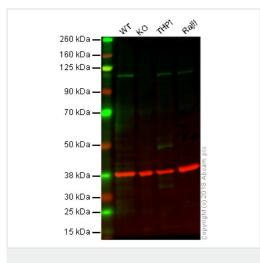
Immunocytochemistry/ Immunofluorescence - Anti-CBL antibody [YE323] - C-terminal (ab32027)

Immunocytochemistry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling CBL with purified ab32027 at 1/50 dilution (4.26 μ g/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 μ g/mL). Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 μ g/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

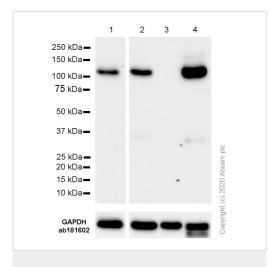


Flow Cytometry (Intracellular) - Anti-CBL antibody [YE323] - C-terminal (ab32027)

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 lgG (Alexa Fluor[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



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



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.

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 lgG H&L (HRP) (<u>ab97051</u>) 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



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- · Valid for 12 months from date of delivery
- · Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

· Guarantee only valid for products bought direct from Abcam or one of our authorized distributors