abcam

Product datasheet

Anti-CEBP Beta antibody [E299] - C-terminal ab32358

敲除验证 重组 RabMAb

7 Abreviews 82 References 13 图像

概述

产品名称	Anti-CEBP Beta抗体[E299] - C-terminal
描述	兔单克隆抗体[E299] to CEBP Beta - C-terminal
宿主	Rabbit
特异性	This antibody is specific for the three CEBPB isoforms (LAP*, LAP and LIP). According to BLAST analysis, the antibody could cross-react with CEBP epsilon (32, 27 and 14kDa, 82% homology) and CEBP alpha (42kDa, 30kDa, 73% homology) in human, mouse and rat. Please be aware that this has not been confirmed experimentally. However, this could explain the background that could possibly be obtained in WB with this antibody. Please contact our Scientific Support if you have any questions.
经测试应用	**适用于:** WB, ICC/IF, IP, Flow Cyt (Intra)
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab274870**)
阳性对照	WB: HeLa, Jurkat, PC-12, NIH/3T3 and MCF7 cell lysate. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa cells. IP: NIH/3T3 cell lysate
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 40% Glycerol, PBS, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	E299
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab32358于以下的经测试应用

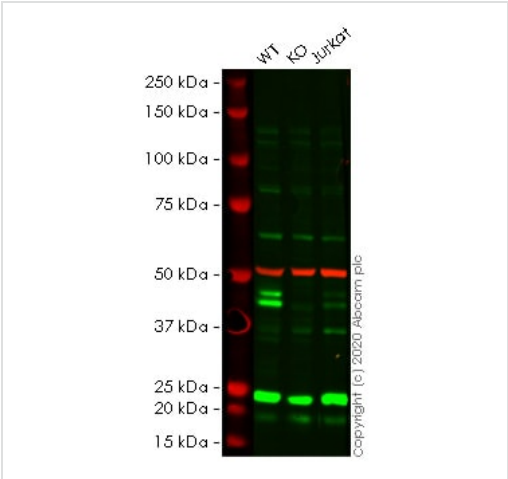
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (3)	1/1000. Predicted molecular weight: 36 kDa.
ICC/IF		1/500. For unpurified use at 1/50 - 1/100
IP		1/30. For unpurified use at 1/30
Flow Cyt (Intra)		1/600. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/1000

靶标

功能	Important transcriptional activator in the regulation of genes involved in immune and inflammatory responses. Specifically binds to an IL-1 response element in the IL-6 gene. NF-IL6 also binds to regulatory regions of several acute-phase and cytokines genes. It probably plays a role in the regulation of acute-phase reaction, inflammation and hemopoiesis. The consensus recognition site is 5'-T[TG]NNGNAA[TG]-3'. Functions in brown adipose tissue (BAT) differentiation.
组织特异性	Expressed at low levels in the lung, kidney and spleen.
序列相似性	Belongs to the bZIP family. C/EBP subfamily. Contains 1 bZIP domain.
结构域	the 9aaTAD motif is a transactivation domain present in a large number of yeast and animal transcription factors.
翻译后修饰	Sumoylated by polymeric chains of SUMO2 or SUMO3.
细胞定位	Nucleus.

图片



Western blot - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

All lanes : Anti-CEBP Beta antibody [E299] - C-terminal (ab32358) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CEBP Beta knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

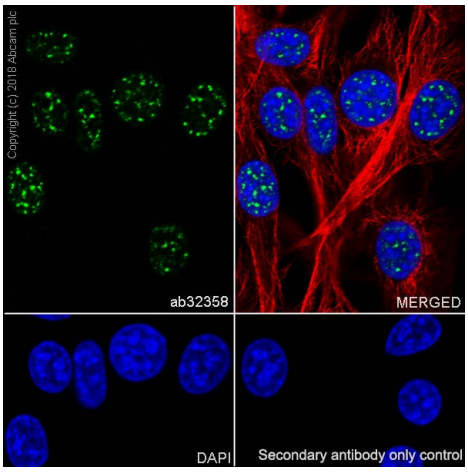
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 36 kDa
Observed band size: 40,45 kDa

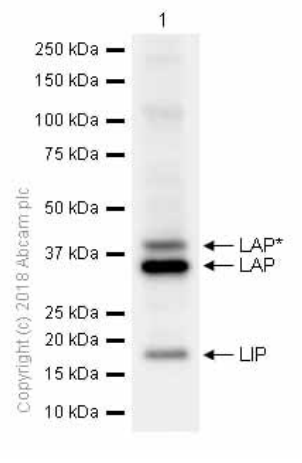
Lanes 1-3: Merged signal (red and green). Green - ab32358 observed at 40 and 45 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab32358 Anti-CEBP Beta antibody [E299] - C-terminal was shown to specifically react with CEBP Beta in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab261771** (knockout cell lysate **ab256874**) was used. Wild-type and CEBP Beta knockout samples were subjected to SDS-PAGE. ab32358 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) 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-CEBP Beta antibody [E299] - C-terminal (ab32358)

Immunocytochemistry/ Immunofluorescence analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labeling CEBP Beta with Purified ab32358 at 1:500 dilution (1.2 µg/ml). Cells were fixed in 100% Methanol. 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 nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



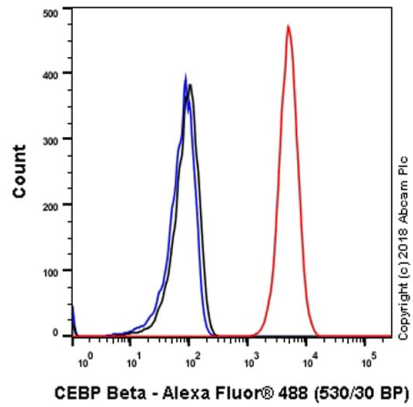
Western blot - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

Anti-CEBP Beta antibody [E299] - C-terminal (ab32358) at 1/1000 dilution + NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates 15 at 15 µg

Secondary

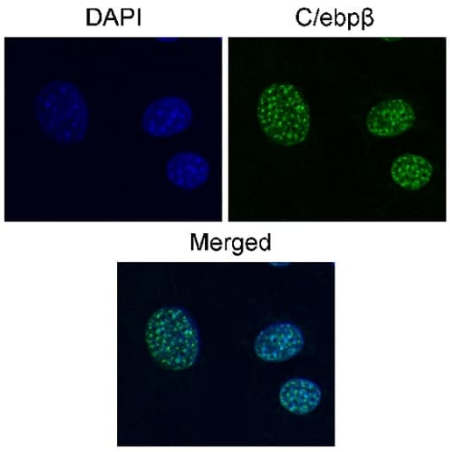
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 36 kDa



Flow Cytometry (Intracellular) - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling CEBP Beta with Purified ab32358 at 1/600 dilution (1µg/ml) (red). Cells were fixed with 80% 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).



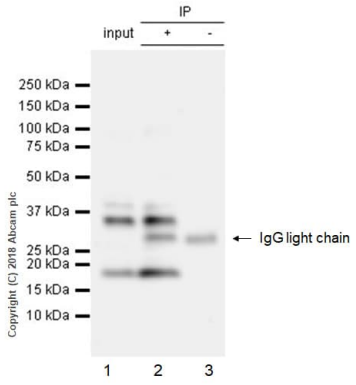
Immunocytochemistry/ Immunofluorescence - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

Sikkeland et al PLoS One. 2013 Jul 10;8(7):e68249. doi: 10.1371/journal.pone.0068249. Print 2013. Fig S1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

3T3-L1 (Mouse embryonic fibroblast cell line) cells were plated on cover slips, grown to post confluency and treated with adipogenic cocktail for 16 h. Cells were washed briefly with phosphate buffered saline (PBS) and fixed in methanol at −20°C for 5 min. Cells were then blocked with 1% BSA for 30 min before incubation with ab32358 at a 1/100 dilution at 4°C overnight and incubated with Alexa Fluor® 488 goat anti-rabbit secondary antibodies (1 : 500) for 1 h at room temperature.

DAPI staining was used for visualizing the nuclei.

Images were acquired with an Olympus FlowView FV1000.



Immunoprecipitation - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

ab32358 (purified) at 1:30 dilution (2µg) immunoprecipitating CEBP Beta in NIH/3T3 whole cell lysate.

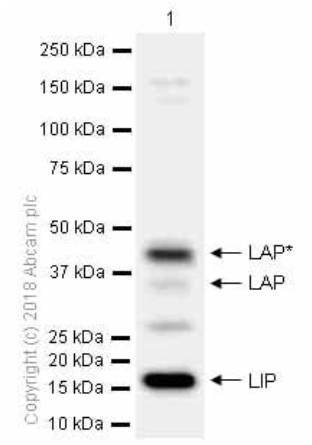
Lane 1 (input): NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate 10µg

Lane 2 (+): ab32358 & NIH/3T3 whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32358 in NIH/3T3 whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



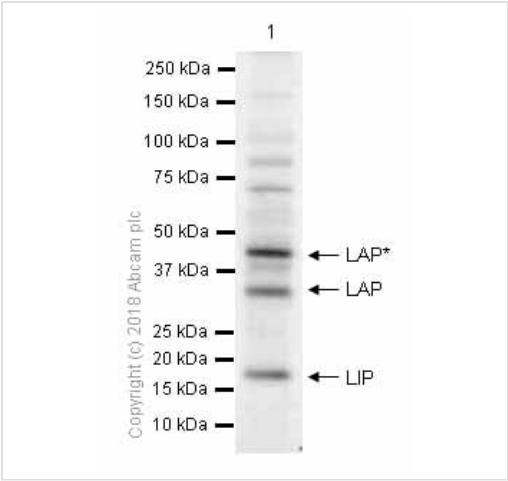
Western blot - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

Anti-CEBP Beta antibody [E299] - C-terminal (ab32358) + MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 36 kDa



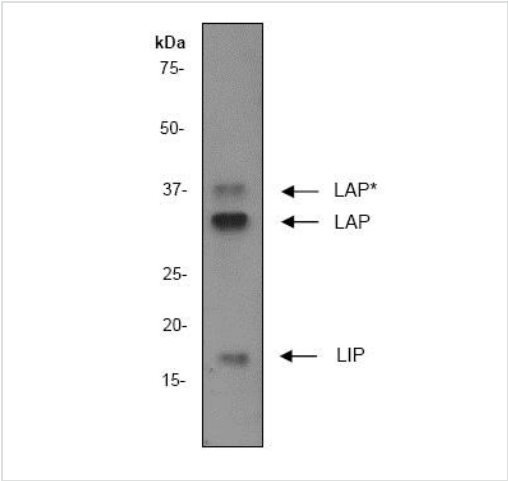
Western blot - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

Anti-CEBP Beta antibody [E299] - C-terminal (ab32358) at 1/1000 dilution + PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates 15ug at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 36 kDa

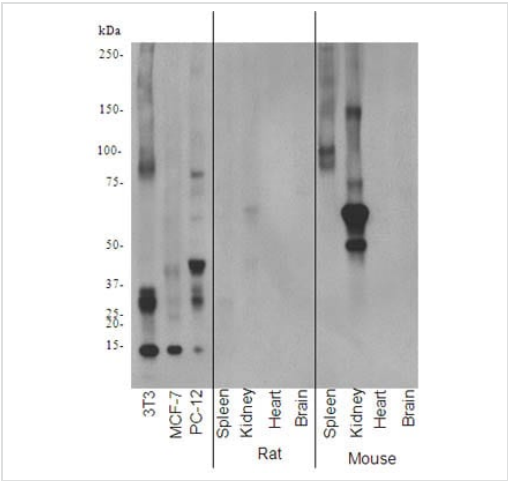


Western blot - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

Anti-CEBP Beta antibody [E299] - C-terminal (ab32358) at 1/1000 dilution + PC-12 (Rat adrenal gland pheochromocytoma cell line) cell lysate.

Predicted band size: 36 kDa

Observed bands
LAP*: 38kDa
LAP: 35kDa
LIP: 20kDa

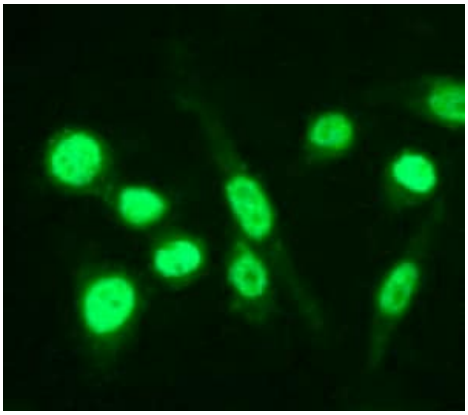


Western blot - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

All lanes : Anti-CEBP Beta antibody [E299] - C-terminal (ab32358) at 1/500 dilution

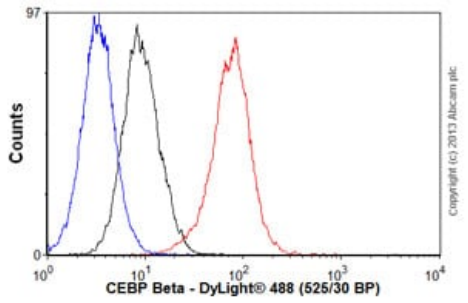
Lane 1 : NIH/3T3 (Mouse embryo fibroblast cell line) cells
Lane 2 : MCF7 (Human breast adenocarcinoma cell line) cells
Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma cell line) cells
Lane 4 : Rat Spleen Lysate
Lane 5 : Rat Kidney Lysate
Lane 6 : Rat Heart Lysate
Lane 7 : Rat Brain Lysate
Lane 8 : Mouse Spleen Lysate
Lane 9 : Mouse Kidney Lysate
Lane 10 : Mouse Heart Lysate
Lane 11 : Mouse Brain Lysate

Predicted band size: 36 kDa



Immunocytochemistry/ Immunofluorescence - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

ab32358, at a 1/50 dilution, staining CEBP Beta in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells by Immunofluorescence.



Flow Cytometry (Intracellular) - Anti-CEBP Beta antibody [E299] - C-terminal (ab32358)

Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with ab32358 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32358, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabeled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Anti-CEBP Beta antibody [E299] - C-terminal

(ab32358)

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