abcam

Product datasheet

Anti-eIF4EBP1 antibody [Y329] ab32024

敲除验证 重组 RabMAb

35 References 11 图像

概述

产品名称	Anti-eIF4EBP1抗体[Y329]
描述	兔单克隆抗体[Y329] to eIF4EBP1
宿主	Rabbit
经测试应用	**适用于:** WB, IHC-P, Flow Cyt (Intra), IP, ICC/IF
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.
阳性对照	WB: HeLa, HAP1, K562 and 293 cell lysates. K-562 and HEK-293 whole cell lysates. Mouse and rat skeletal muscle lysate. Rat L6 whole cell lysate. IHC-P: Human colon cancer tissue. Rat and mouse spleen tissues. Flow Cyt (intra): HAP1-WT, HEK-293, and HT1080 cells. IP: HEK-293 whole cell lysate. ICC/IF: HeLa cells.
常规说明	Binding of eIF4EBP1 to eIF4E is reversible and is dependent on the phosphorylation status of eIF4EBP1. Non phosphorylated eIF4EBP1 will bind strongly to eIF4E while, the phosphorylated form will not. Akt, TOR, MAP kinase, S6 kinase, and Cdc2 are known kinases capable of inactivating eIF4EBP1 binding to eIF4E by phosphorylating either threonines 35, 45, 69 or serine 64. Although, not all phosphorylation events equally block the eIF4EBP1-eIF4E interaction. 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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
Primary antibody说明	Binding of eIF4EBP1 to eIF4E is reversible and is dependent on the phosphorylation status of eIF4EBP1. Non phosphorylated eIF4EBP1 will bind strongly to eIF4E while, the phosphorylated form will not. Akt, TOR, MAP kinase, S6 kinase, and Cdc2 are known kinases capable of inactivating eIF4EBP1 binding to eIF4E by phosphorylating either threonines 35, 45, 69 or serine 64. Although, not all phosphorylation events equally block the eIF4EBP1-eIF4E interaction
克隆	单克隆
克隆编号	Y329
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab32024**于以下的**经测试应用

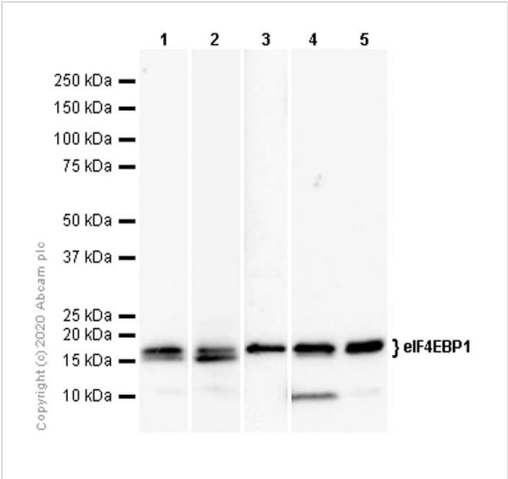
"应用说明"**部分 下显示的**仅为**推荐的起始稀释度**;实际**最佳的稀释度/浓度应由使用者检定。**

应用	Ab评论	说明
WB		1/2000. Detects a band of approximately 17 kDa (predicted molecular weight: 13 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/20.
IP		1/20.
ICC/IF		1/500.

靶标

功能	Regulates eIF4E activity by preventing its assembly into the eIF4F complex. Mediates the regulation of protein translation by hormones, growth factors and other stimuli that signal through the MAP kinase and mTORC1 pathways.
序列相似性	Belongs to the eIF4E-binding protein family.
翻译后修饰	Phosphorylated on serine and threonine residues in response to insulin, EGF and PDGF. Phosphorylation at Thr-37, Thr-46, Ser-65 and Thr-70 is regulated by mTORC1. Phosphorylated upon DNA damage, probably by ATM or ATR.

图片



Western blot - Anti-eIF4EBP1 antibody [Y329] (ab32024)

All lanes : Anti-eIF4EBP1 antibody [Y329] (ab32024) at 1/2000 dilution (Purified)

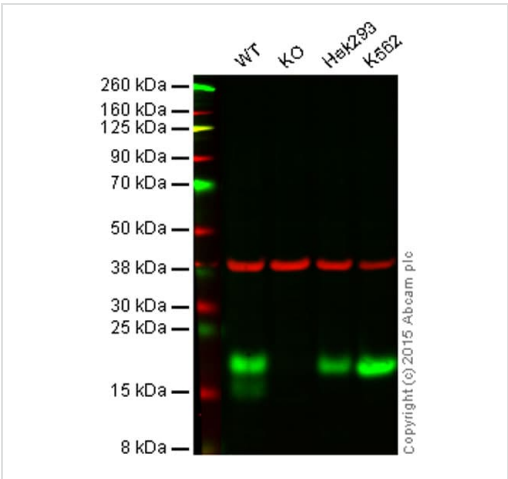
Lane 1 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate
Lane 2 : K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate
Lane 3 : Mouse skeletal muscle lysate
Lane 4 : Rat skeletal muscle lysate
Lane 5 : L6 (Rat skeletal muscle myoblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 13 kDa
Observed band size: 18 kDa

The molecular weights observed are consistent with what has been described in the literatures (PMID: 28613975, 20890458 and 27358481).



Western blot - Anti-eIF4EBP1 antibody [Y329] (ab32024)

All lanes : Anti-eIF4EBP1 antibody [Y329] (ab32024)

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : eIF4EBP1 knockout HAP1 cell lysate
Lane 3 : HEK293 cell lysate
Lane 4 : K562 cell lysate

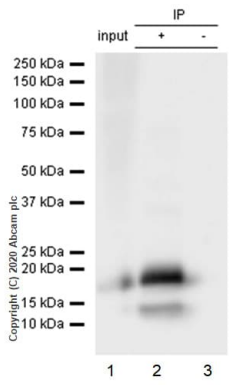
Lysates/proteins at 20 µg per lane.

Predicted band size: 13 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab32024 observed at 20 kDa. Red - loading control, **ab8245**, observed at 37

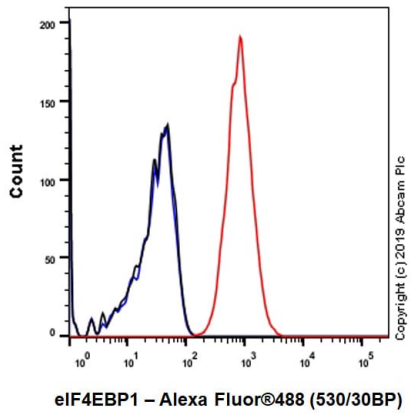
kDa.

ab32024 was shown to specifically react with eIF4EBP1 in wild-type Hap1 cells. Wild-type and eIF4EBP1 knockout samples were subjected to SDS-PAGE. ab32024 and **ab8245** (loading control to GADPH) were diluted 1/5000 and 1/2000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.



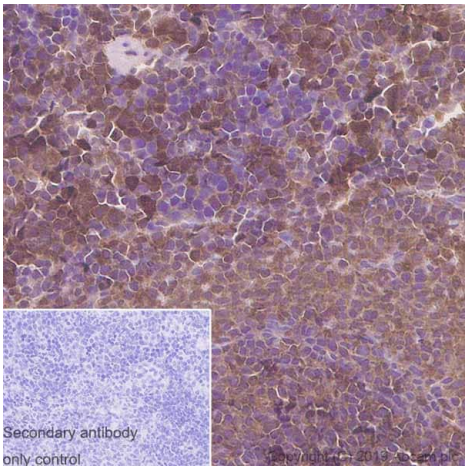
Immunoprecipitation - Anti-eIF4EBP1 antibody [Y329] (ab32024)

Purified ab32024 at 1/20 dilution (2µg) immunoprecipitating eIF4EBP1 in HEK-293 whole cell lysate.
Lane 1 (input): HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab32024 + HEK-293 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32024 in HEK-293 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 15-20 kDa



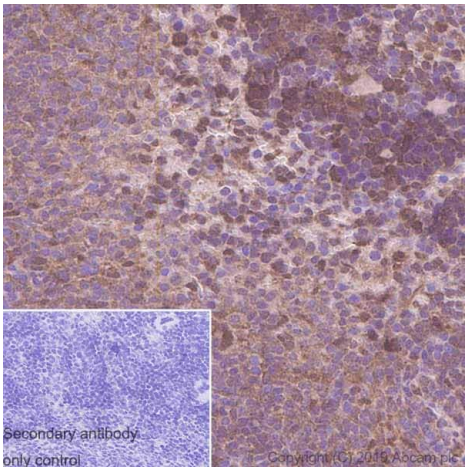
Flow Cytometry (Intracellular) - Anti-eIF4EBP1 antibody [Y329] (ab32024)

Intracellular Flow Cytometry analysis of HEK-293 (Human embryonic kidney epithelial cell) cells labeling eIF4EBP1 with purified ab32024 at 1/20 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).



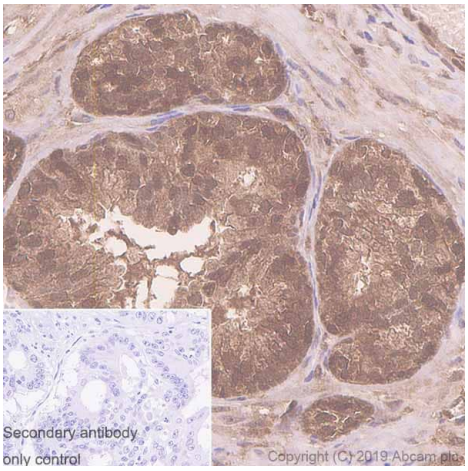
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eIF4EBP1 antibody [Y329] (ab32024)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat spleen tissue sections labeling eIF4EBP1 with purified ab32024 at 1/200 dilution (0.53 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



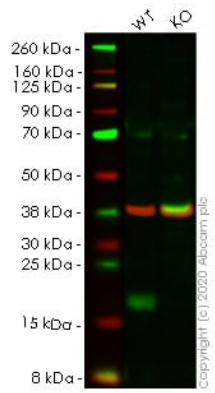
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eIF4EBP1 antibody [Y329] (ab32024)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse spleen tissue sections labeling eIF4EBP1 with purified ab32024 at 1/200 dilution (0.53 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eIF4EBP1 antibody [Y329] (ab32024)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon cancer tissue sections labeling eIF4EBP1 with purified ab32024 at 1/200 dilution (0.53 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-eIF4EBP1 antibody [Y329] (ab32024)

All lanes : Anti-eIF4EBP1 antibody [Y329] (ab32024) at 1/5000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : EIF4EBP1 knockout HeLa cell lysate

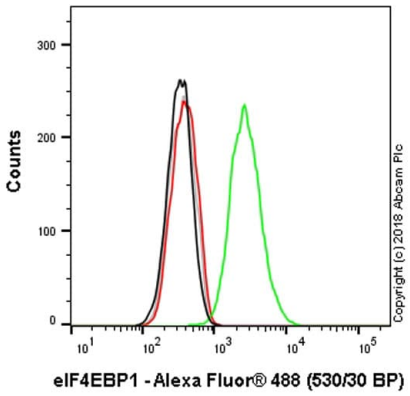
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 13 kDa
Observed band size: 13 kDa

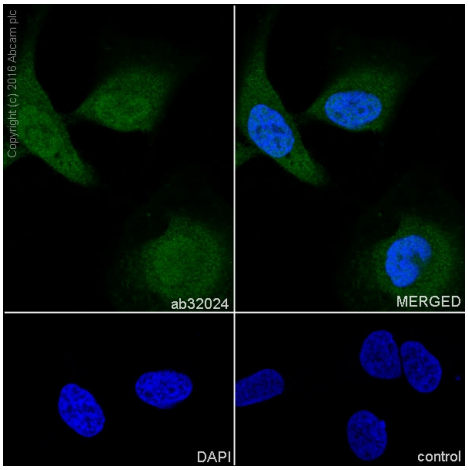
Lanes 1-2: Merged signal (red and green). Green - ab32024 observed at 13 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab32024 Anti-eIF4EBP1 antibody [Y329] was shown to specifically react with eIF4EBP1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab264784** (knockout cell lysate **ab257146**) was used. Wild-type and eIF4EBP1 knockout samples were subjected to SDS-PAGE. ab32024 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 5000 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.



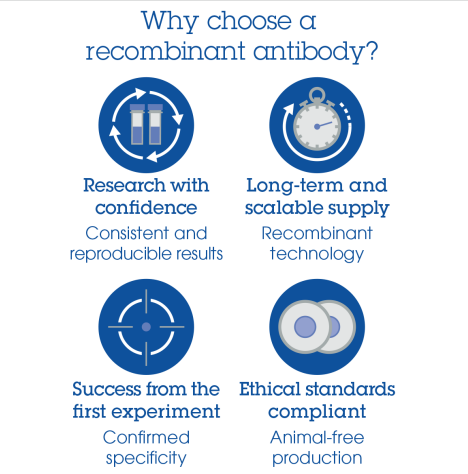
Flow Cytometry (Intracellular) - Anti-eIF4EBP1 antibody [Y329] (ab32024)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-EIF4EBP1 knockout cells (red line) stained with ab32024. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab32024, 0.1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-EIF4EBP1 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This antibody can also be used in HAP1 cells fixed with 4% formaldehyde (10 min) , permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-eIF4EBP1 antibody [Y329] (ab32024)

Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) labeling eIF4EBP1 with Purified ab32024 at 1/500 dilution (5 µg/ml). Cells were fixed with 4% PFA and permeabilized with 0.1% tritonX-100. **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) at 1/1000 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI. PBS was used instead of the primary antibody as the negative control.



Anti-eIF4EBP1 antibody [Y329] (ab32024)

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