abcam

ab32024

Anti-eIF4EBP1 antibody [Y329]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	Y329
Purification technique	Affinity purification Protein A
Concentration	0.106 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Mouse
Dilution info	1/100 - 1/250
Notes	-

Species	Rat

Dilution info	1/100 - 1/250
Notes	-

Species	Human
Dilution info	1/100 - 1/250
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Human
Dilution info	1/20
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/2000
Notes	-

Species	Rat
Dilution info	1/2000
Notes	-

Species	Human
Dilution info	1/2000
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information EIF4EBP1

Function Repressor of translation initiation that regulates EIF4E activity by preventing its assembly into the eIF4F complex: hypophosphorylated form competes with EIF4G1/EIF4G3 and strongly binds to EIF4E, leading to repress translation. In contrast, hyperphosphorylated form dissociates from EIF4E, allowing interaction between EIF4G1/EIF4G3 and EIF4E, leading to initiation of translation. Mediates the regulation of protein translation by hormones, growth factors and other stimuli that signal through the MAP kinase and mTORC1 pathways.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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.

Patented technology

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

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	EIF4EBP1 also known as 4EBP1 or p4EBP1 is an important protein that acts as a translational repressor by binding to eIF4E therefore inhibiting cap-dependent translation. The molecular weight of 4EBP1 is approximately 12 kDa. This protein is ubiquitously expressed in various tissues indicating its widespread involvement in cellular functions. eIF4EBP1 can be detected and quantified using techniques like

	the eIF4EBP1 ELISA making it a common target in research for its role in translation regulation.
Biological function summary	EIF4EBP1 plays an important role in regulating cell growth and proliferation by modulating protein synthesis. It is a part of the eIF4F complex which is responsible for the initiation of mRNA translation. When hypophosphorylated eIF4EBP1 binds tightly to eIF4E and prevents the assembly of the active eIF4F complex leading to reduced translation initiation of mRNAs involved in growth and survival. This regulation is important in conditions where cells need to adapt to metabolic stress or external signals.
Pathways	The mammalian target of rapamycin (mTOR) pathway regulates eIF4EBP1 through phosphorylation. In response to growth signals mTOR phosphorylates eIF4EBP1 causing the release of eIF4E and allowing mRNA translation to proceed. This interaction links eIF4EBP1 to the PI3K/AKT/mTOR signaling pathway which influences cell cycle progression and survival. Related proteins in this pathway include ribosomal protein S6 kinase (S6K1) which is also phosphorylated by mTOR to promote protein synthesis.
Associated diseases and disorders	Deregulation of eIF4EBP1 has been implicated in cancer and neurological disorders. In cancer hyperactive mTOR signaling can lead to excessive phosphorylation of 4EBP1 decreasing its ability to inhibit eIF4E and enhancing translation of oncogenic proteins. In neurological disorders imbalanced eIF4EBP1 activity can disrupt synaptic plasticity and memory formation. Proteins such as p70S6K which are also part of the mTOR pathway share connections with eIF4EBP1 in these pathological conditions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

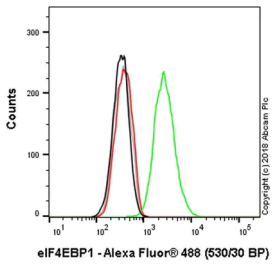
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

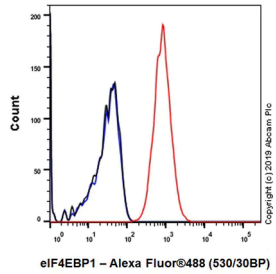
10 product images



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

EIF4EBP1 Flow Cytometry (Intracellular) staining using rabbit Anti-eIF4EBP1 antibody

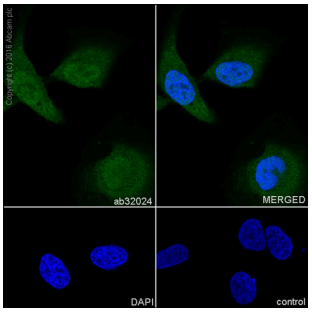
Overlay histogram showing HAP1 wildtype (green line) and HAP1-EIF4EBP1 knockout cells (red line) stained with ab32024. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block 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.



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

EIF4EBP1 Flow Cytometry (Intracellular) staining using rabbit Anti-eIF4EBP1 antibody

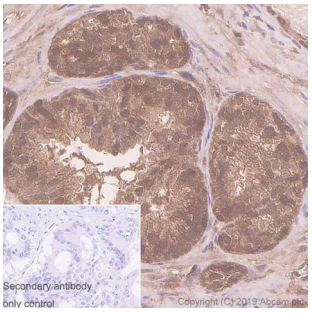
Intracellular Flow Cytometry analysis of HEK-293 (Human embryonic kidney epithelial cell) cells labeling eIF4EBP1 with purified ab32024 at 1/20 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) 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).



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

EIF4EBP1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-eIF4EBP1 antibody

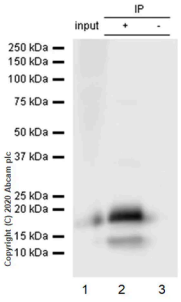
Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) labeling eIF4EBP1 with Purified ab32024 at 1/500 dilution (5 µg/ml). Cells were fixed with 4% PFA and permeabilized with 0.1% tritonX-100. ab150077 Goat anti rabbit IgG(Alexa Fluor® 488) at 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.



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

EIF4EBP1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-eIF4EBP1 antibody

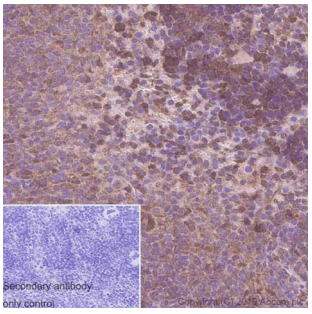
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon cancer tissue sections labeling eIF4EBP1 with purified ab32024 at 1/200 dilution (0.53 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection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



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

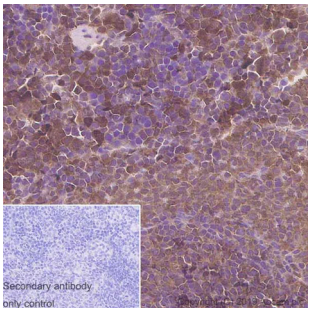
Predicted band size: 13 kDa



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

EIF4EBP1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-eIF4EBP1 antibody

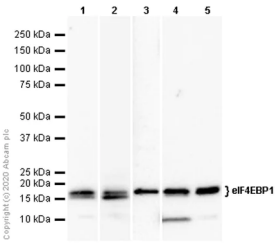
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse spleen tissue sections labeling eIF4EBP1 with purified ab32024 at 1/200 dilution (0.53 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection 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)

EIF4EBP1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-eIF4EBP1 antibody

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.



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

The molecular weights observed are consistent with what has been described in the literatures (PMID: 28613975, 20890458 and 27358481).
All lanes:
Western blot - Anti-eIF4EBP1 antibody [Y329] (ab32024) at 1/2000 dilution

Lane 1:
HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 2:
K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate at 20 µg

Lane 3:
Mouse skeletal muscle lysate at 20 µg

Lane 4:
Rat skeletal muscle lysate at 20 µg

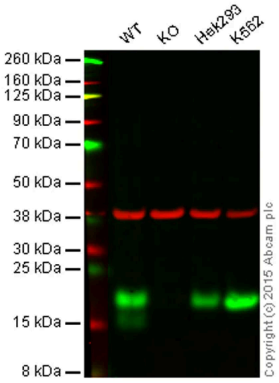
Lane 5:
L6 (Rat skeletal muscle myoblast) whole cell lysate at 20 µg

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



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

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

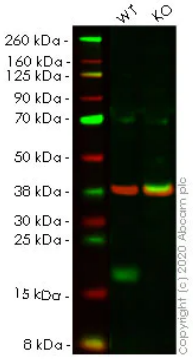
Lane 1:
Wild-type HAP1 cell lysate at 20 µg

Lane 2:
eIF4EBP1 knockout HAP1 cell lysate at 20 µg

Lane 3:
HEK293 cell lysate at 20 µg

Lane 4:
K562 cell lysate at 20 µg

Predicted band size: 13 kDa



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

EIF4EBP1 Western blot staining using rabbit Anti-eIF4EBP1 antibody

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.

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

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human EIF4EBP1 knockout HeLa cell lysate (ab257146) at 20 µg

Secondary

Lanes 1 - 2:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution

Lanes 1 - 3:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 13 kDa

Observed band size: 13 kDa, 37 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.