# abcam

## Product datasheet

## Anti-elF4E antibody [Y448] ab33766



重组 RabMAb

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#### 概述

产品名称 Anti-elF4E抗体[Y448]

描述 兔单克隆抗体[Y448] to eIF4E

宿主 Rabbit

特异性 The antibody detects a band on western blot of approximately 28 kDa.

经测试应用 适用于: Flow Cyt (Intra), ICC/IF, IP, WB, IHC-P

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide within Human elF4E aa 1-100 (N terminal). The exact sequence is proprietary.

阳性对照 WB: 293, HEK-293, and MCF7 cell lysates, Human brain tissue lysate IHC-P: human breast

carcinoma, Human cervical carcinoma and Mouse stomachICC/IF: RAW 264.7 cells Flow Cyt

(intra): HEK-293 cells

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

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: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

纯度 Protein A purified

克隆 单克隆 克隆编号 Y448

同种型 lgG

#### 应用

### The Abpromise guarantee Abpromise™承诺保证使用ab33766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   |
|------------------|----------------------|--|
| Flow Cyt (Intra) |                      | Use 1µg for 10 <sup>6</sup> cells.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.                                    |
| ICC/IF           |                      | 1/250 - 1/500.   |
| IP               | <b>★</b> 市 市 市 市 (1) | 1/20.  |
| WB               | <b>★★★★★</b> (6)     | 1/1000. Detects a band of approximately 30 kDa (predicted molecular weight: 25 kDa). <b>For unpurified use at 1/500</b>  |
| IHC-P            |                      | 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See <b>IHC antigen retrieval protocols</b> . |

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## 功能 Its translation stimulation activity is repressed by binding to the complex CYFIP1-FMR1 (By

similarity). Recognizes and binds the 7-methylguanosine-containing mRNA cap during an early step in the initiation of protein synthesis and facilitates ribosome binding by inducing the unwinding of the mRNAs secondary structures. Component of the CYFIP1-EIF4E-FMR1 complex which binds to the mRNA cap and mediates translational repression. In the CYFIP1-EIF4E-FMR1

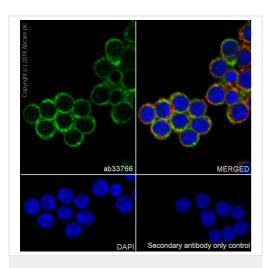
complex this subunit mediates the binding to the mRNA cap.

**序列相似性** Belongs to the eukaryotic initiation factor 4E family.

翻译后修饰 Phosphorylation increases the ability of the protein to bind to mRNA caps and to form the eIF4F

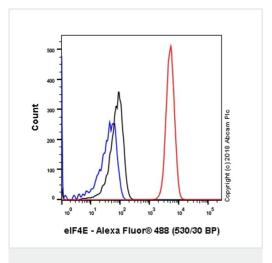
complex.

## 图片



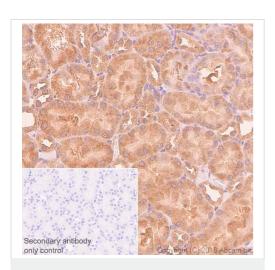
Immunocytochemistry/ Immunofluorescence - AntielF4E antibody [Y448] (ab33766)

Immunocytochemistry/ Immunofluorescence analysis of RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) cells labeling elF4E with Purified ab33766 at 1:500 dilution (0.3 µg/ml). Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor  $^{\otimes}$  594) 1:200 (2.5 µg/ml). Goat anti rabbit lgG (Alexa Fluor  $^{\otimes}$  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.



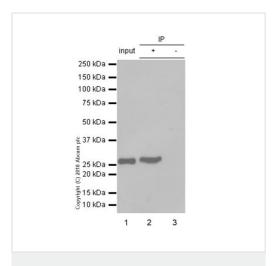
Flow Cytometry (Intracellular) - Anti-elF4E antibody [Y448] (ab33766)

Intracellular Flow Cytometry analysis of HEK-293 (Human embryonic kidney epithelial cell) cells labeling elF4E with Purified ab33766 at 1/200 dilution (1µg/ml) (red). Cells were fixed with 80% Methanol. A Goat anti rabbit lgG (Alexa Fluorr<sup>®</sup> 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).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-elF4E antibody [Y448] (ab33766)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat kidney tissue sections labeling eIF4E with Purified ab33766 at 1:100 dilution (1.33 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10mins. 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-elF4E antibody [Y448] (ab33766)

ab33766 (purified) at 1:20 dilution (0.6µg) immunoprecipitating eIF4E in HEK-293 whole cell lysate.

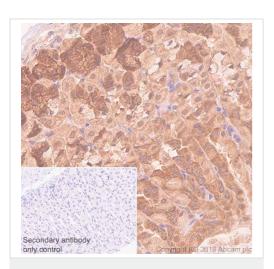
Lane 1 (input): HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10µg

Lane 2 (+): ab33766 & HEK-293 whole cell lysate

Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab33766 in HEK-293 whole cell lysate

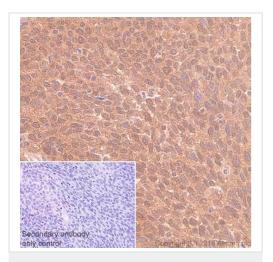
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:10,000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



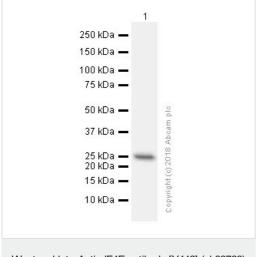
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-elF4E antibody [Y448] (ab33766)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse stomach tissue sections labeling eIF4E with Purified ab33766 at 1:100 dilution (1.33 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10mins. 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 paraffinembedded sections) - Anti-elF4E antibody [Y448] (ab33766)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human cervical carcinoma tissue sections labeling eIF4E with Purified ab33766 at 1:100 dilution (1.33 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10mins. 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.



Anti-elF4E antibody [Y448] (ab33766) at 1/1000 dilution (Purified) + Human brain lysates at 15 µg

### Secondary

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

**Predicted band size:** 25 kDa **Observed band size:** 25 kDa

Western blot - Anti-elF4E antibody [Y448] (ab33766)

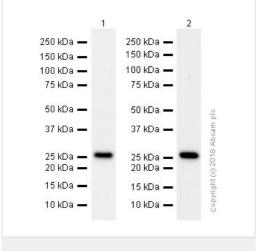
Blocking and dilutin buffer and concentration: 5% NFDM/TBST

All lanes: Anti-elF4E antibody [Y448] (ab33766) at 1/5000 dilution

**Lane 1**: HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates

**Lane 2 :** MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.



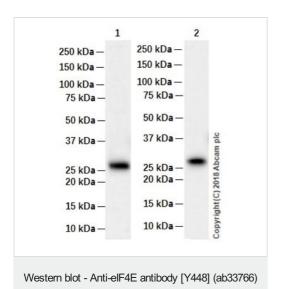
Western blot - Anti-elF4E antibody [Y448] (ab33766)

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

**Predicted band size:** 25 kDa **Observed band size:** 25 kDa

Blocking and dilutin buffer and concentration: 5% NFDM/TBST



All lanes: Anti-elF4E antibody [Y448] (ab33766) at 0.025 µg/ml

Lane 1: Raw264.7 (Mouse abelson murine leukemia virus-induced tumor) whole cell lysate

Lane 2: C6 (Rat glioma) whole cell lysate

Lysates/proteins at 20 µg per lane.

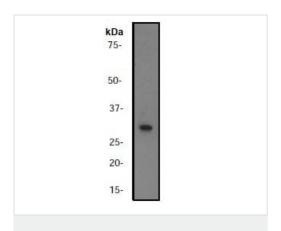
### Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000

Predicted band size: 25 kDa

Blocking and diluting buffer: 5% NFDM/TBST.

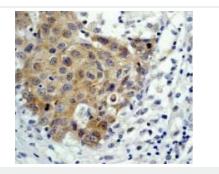
Exposure time - Lane 1: 160 seconds Lane 2: 70 seconds



Western blot - Anti-elF4E antibody [Y448] (ab33766)

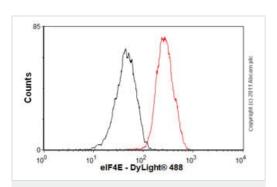
Anti-elF4E antibody [Y448] (ab33766) at 1/500 dilution + 293 cell lysate

Predicted band size: 25 kDa Observed band size: 30 kDa



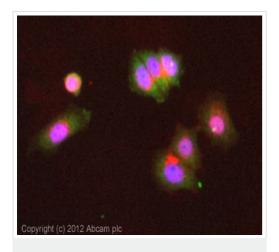
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-elF4E antibody [Y448] (ab33766)

This image shows human breast carcinoma stained with ab33766



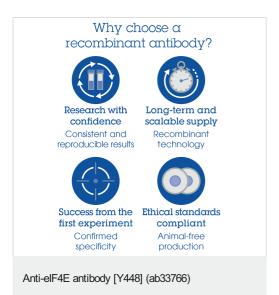
Flow Cytometry (Intracellular) - Anti-elF4E antibody [Y448] (ab33766)

Overlay histogram showing HEK293 cells stained with ab33766 (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. The cells were then incubated with the antibody (ab33766, 1 $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (1 $\mu$ g/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunocytochemistry/ Immunofluorescence - AntieIF4E antibody [Y448] (ab33766)

ICC/IF image of ab33766 stained MCF7 cells. The cells were 4% formaldehyde (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab33766, 10μg/ml) overnight at +4°C. The secondary antibody (green) was ab96899 Dylight 488 goat anti-rabbit lgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43μM.



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