abcam

Product datasheet

# Anti-eIF4E antibody [Y448] ab33766

Recombinant RabMAb

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## Overview

| | |
|---|---|
| **Product name** | Anti-eIF4E antibody [Y448] |
| **Description** | Rabbit monoclonal [Y448] to eIF4E |
| **Host species** | Rabbit |
| **Specificity** | The antibody detects a band on western blot of approximately 28 kDa. |
| **Tested applications** | **Suitable for:** Flow Cyt (Intra), ICC/IF, IP, WB, IHC-P |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human |
| **Immunogen** | Synthetic peptide within Human eIF4E aa 1-100 (N terminal). The exact sequence is proprietary. |
| **Positive control** | 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 |
| **General notes** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>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

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|---|---|
| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles. |
| **Storage buffer** | pH: 7.20<br>Preservative: 0.01% Sodium azide<br>Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | Y448 |

| | |
|---|---|
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab33766 in the following tested applications.

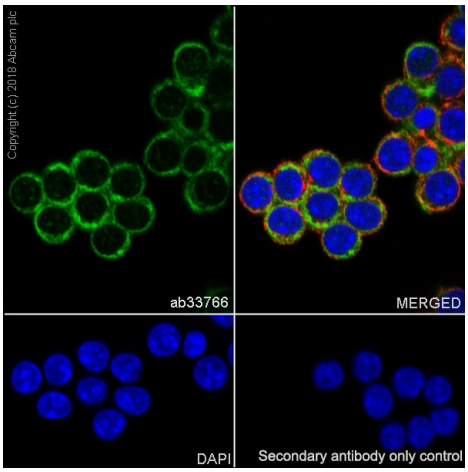
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| **Flow Cyt (Intra)** | | Use 1µg for $10^6$ cells.<br>**ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |
| **ICC/IF** | | 1/250 - 1/500. |
| **IP** | ★☆☆☆☆ **(1)** | 1/20. |
| **WB** | ★★★★★ **(6)** | 1/1000. Detects a band of approximately 30 kDa (predicted molecular weight: 25 kDa). **For unpurified use at 1/500** |
| **IHC-P** | | 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. |

## Target

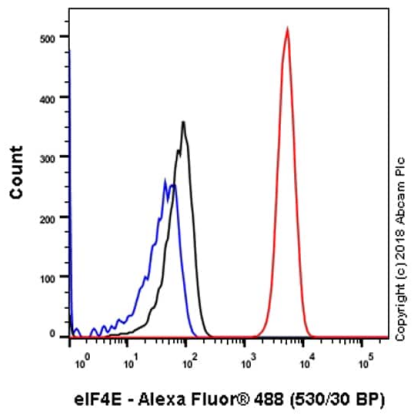
| | |
|---|---|
| **Function** | Its translation stimulation activity is repressed by binding to the complex CYFIP1-FMR1 (By similarity). Recognizes and binds the 7-methylguanosine-containing mRNA cap during an early step in the initiation of protein synthesis and facilitates ribosome binding by inducing 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. |
| **Sequence similarities** | Belongs to the eukaryotic initiation factor 4E family. |
| **Post-translational modifications** | Phosphorylation increases the ability of the protein to bind to mRNA caps and to form the eIF4F complex. |

## Images



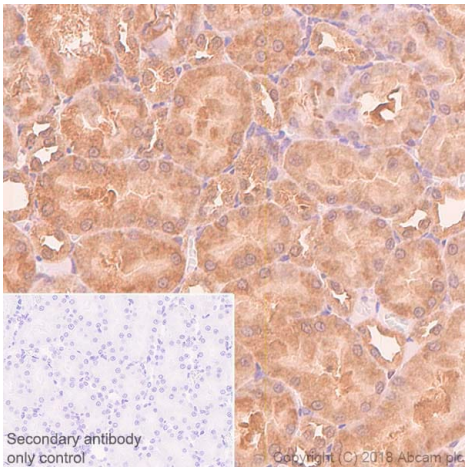

Immunocytochemistry/ Immunofluorescence - Anti-eIF4E antibody [Y448] (ab33766)

Immunocytochemistry/ Immunofluorescence analysis of RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) cells labeling eIF4E 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® 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.



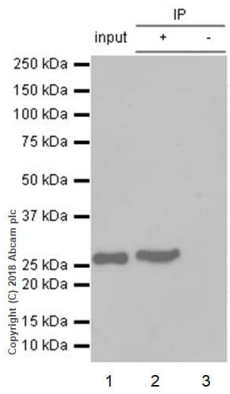

Flow Cytometry (Intracellular) - Anti-eIF4E antibody [Y448] (ab33766)

Intracellular Flow Cytometry analysis of HEK-293 (Human embryonic kidney epithelial cell) cells labeling eIF4E with Purified ab33766 at 1/200 dilution (1µg/ml) (red). Cells were fixed with 80% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 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).



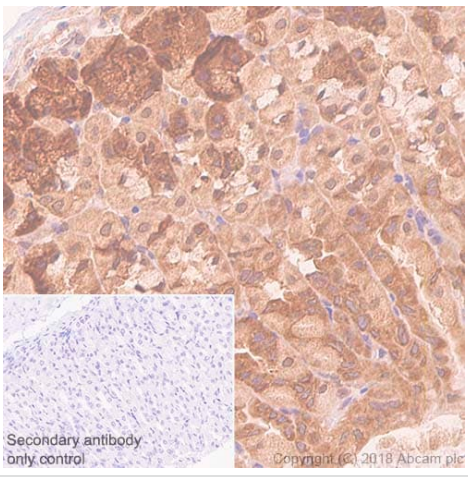

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eIF4E antibody [Y448] (ab33766)

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.



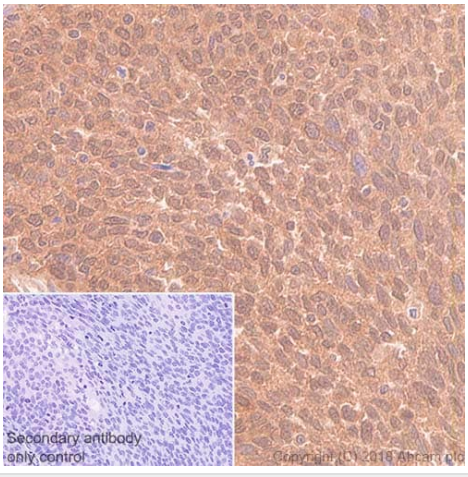

Immunoprecipitation - Anti-eIF4E antibody [Y448] (ab33766)

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 IgG (**ab172730**) 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 paraffin-embedded sections) - Anti-eIF4E 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 paraffin-embedded sections) - Anti-eIF4E 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.


Western blot - Anti-eIF4E antibody [Y448] (ab33766)

Anti-eIF4E antibody [Y448] (ab33766) at 1/1000 dilution (Purified) + Human brain lysates at 15 µg

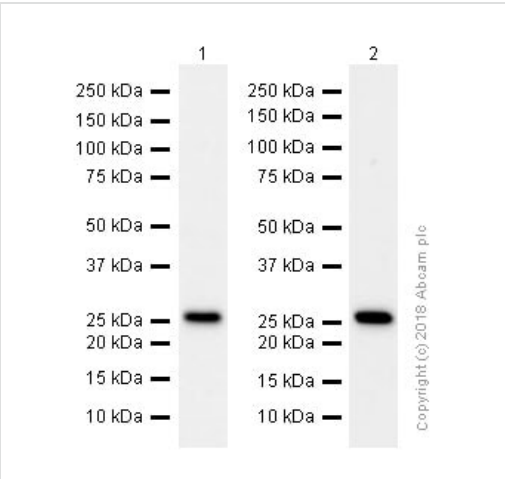
**Secondary**

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

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




Western blot - Anti-eIF4E antibody [Y448] (ab33766)

**All lanes :** Anti-eIF4E 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.

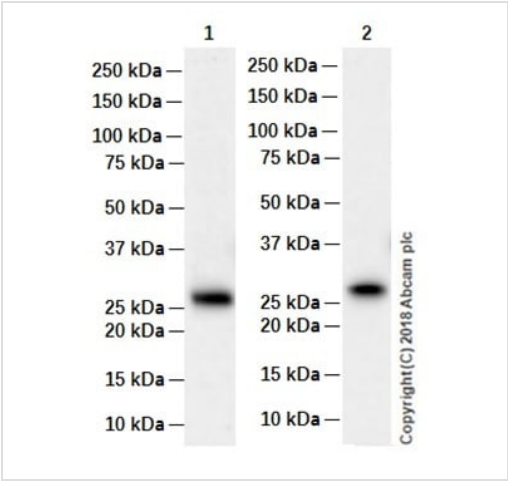
**Secondary**

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

**Predicted band size:** 25 kDa

**Observed band size:** 25 kDa

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




Western blot - Anti-eIF4E antibody [Y448] (ab33766)

**All lanes :** Anti-eIF4E 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

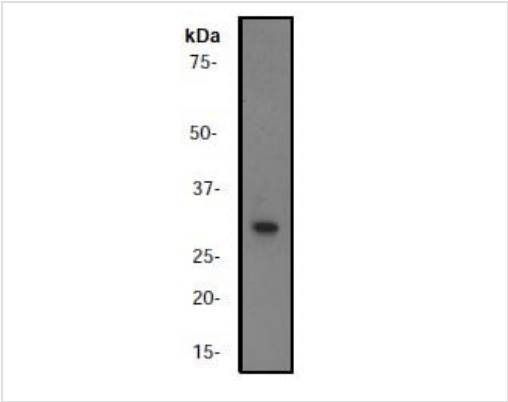
Lysates/proteins at 20 µg per lane.

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

**Predicted band size:** 25 kDa

Blocking and diluting buffer: 5% NFDM/TBST.

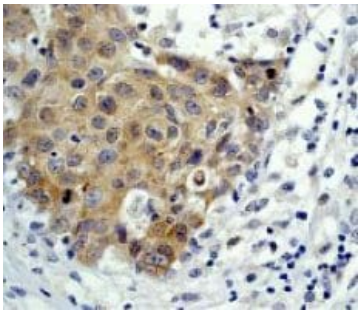
Exposure time - Lane 1: 160 seconds
Lane 2: 70 seconds




Western blot - Anti-eIF4E antibody [Y448] (ab33766)

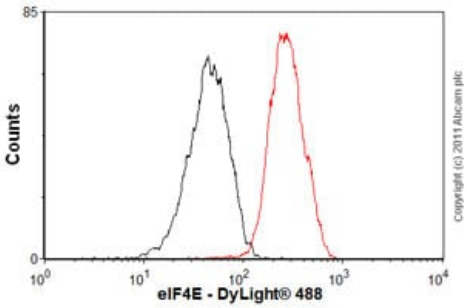
Anti-eIF4E antibody [Y448] (ab33766) at 1/500 dilution + 293 cell lysate

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



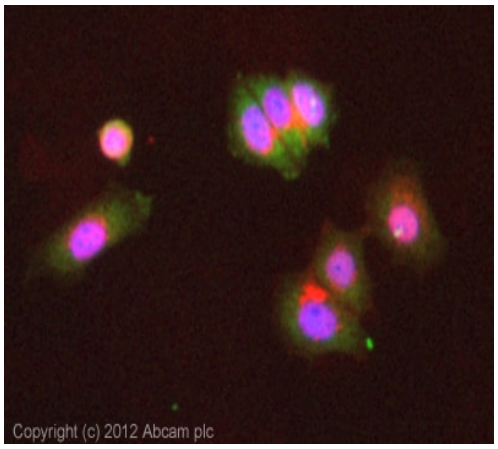
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eIF4E antibody [Y448] (ab33766)

This image shows human breast carcinoma stained with ab33766



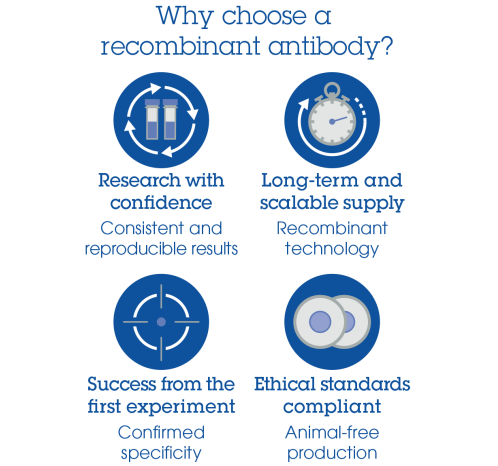

Flow Cytometry (Intracellular) - Anti-eIF4E antibody [Y448] (ab33766)

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, 1µg/1x$10^6$ cells) 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 (1µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.




Immunocytochemistry/ Immunofluorescence - Anti-eIF4E 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 IgG (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.




Anti-eIF4E antibody [Y448] (ab33766)

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