abcam

Product datasheet

Anti-eIF4E (phospho S209) antibody [EP2151Y] ab76256

Recombinant RabMAb

★★★★★ 2 Abreviews 49 References 11 Images

Overview

Product name	Anti-eIF4E (phospho S209) antibody [EP2151Y]
Description	Rabbit monoclonal [EP2151Y] to eIF4E (phospho S209)
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IP, IHC-P, Dot blot
Species reactivity	**Reacts with:** Mouse, Rat, Human, Pig
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: 293 cell lysate treated with alkaline phosphatase and HEK293 cell lysate treated with Dexamethasone. IHC-P: human breast carcinoma tissue. ICC/IF: HEK293 cells.
General notes	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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP2151Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab76256 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
ICC/IF		1/500.
WB		1/1000 - 1/100000. Detects a band of approximately 25 kDa (predicted molecular weight: 25 kDa).
IP		1/40 - 1/60.
IHC-P	★★★★★ (1)	1/50 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Dot blot		1/1000.

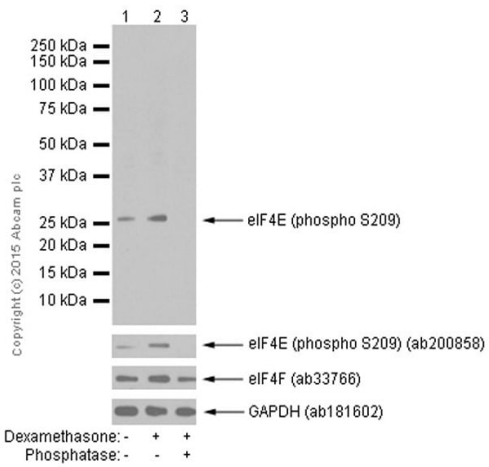
Target

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



Western blot - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

All lanes : Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/100000 dilution (purified)

Lane 1 : Untreated HEK293 whole cell lysate
Lane 2 : HEK293 cells treated with 10uM dexamethasone for 1 hour whole cell lysate
Lane 3 : HEK293 cells treated with 10uM dexamethasone for 1 hour whole cell lysate.The membrane was then incubated with alkaline phosphatase.

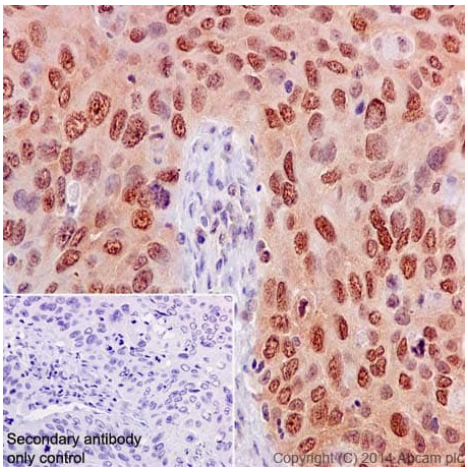
Lysates/proteins at 10 µg per lane.

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

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

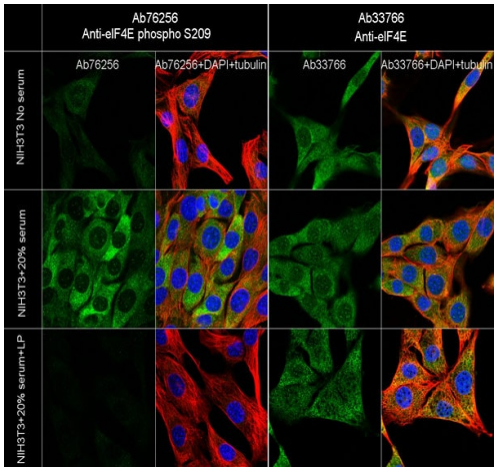
Exposure time: 30 seconds

Blocking buffer and concentration 2% BSA/TBST.
Diluting buffer and concentration 2% BSA/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labelling eIF4E with purified ab76256 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a goat anti-rabbit IgG H&L (HRP) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



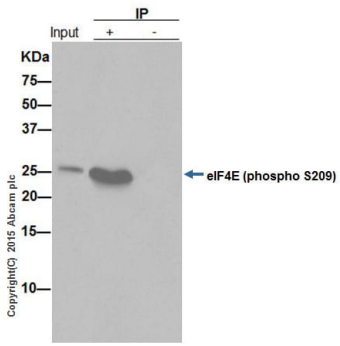
Immunocytochemistry/ Immunofluorescence - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

Immunocytochemistry/Immunofluorescence analysis of untreated, 20% serum treated and 20% serum + LP treated NIH/3T3 cells labelling eIF4E (phospho S209) with ab76256 (left) and eIF4E with **ab33766** (right) both at a dilution of 1/500.

Cells were fixed with 100% methanol. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

The image shows increased cytoplasmic staining after 20% serum treatment on NIH3T3 cells when compared with no serum treated cells. The LP treatment decreased the increased cytoplasmic staining caused by 20% serum.

ab33766 was used as a Pan control for ab76256. The results showed cytoplasmic staining on no serum, 20% serum and 20% serum +LP treated NIH3T3 cells.

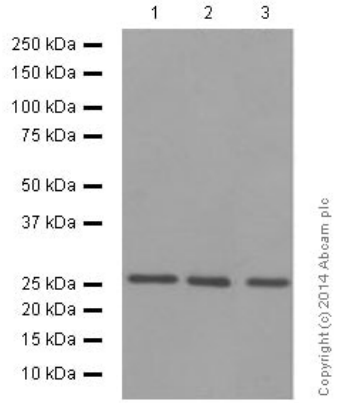


Immunoprecipitation - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

ab76256 (purified) at 1/40 immunoprecipitating eIF4E (phospho S209) in HEK293 whole cell lysate. 10 ug of cell lysate was present in the input. For western blotting, a HRP-conjugated Veriblot for IP Detection Reagent (**ab131366**) (1/1,500) was used for detection. A rabbit monoclonal IgG (**ab172730**) was used intead of **ab128913** as a negative control (Lane 3).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

All lanes : Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/1000 dilution (purified)

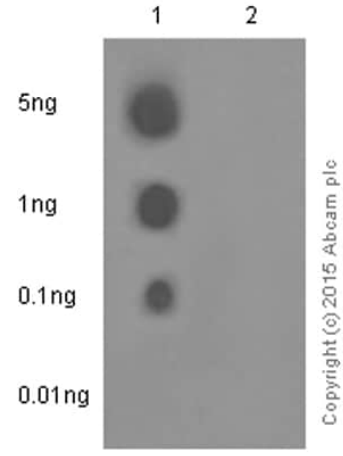
Lane 1 : Mouse spleen lysate
Lane 2 : Rat brain lysate
Lane 3 : Pig heart lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 25 kDa

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.

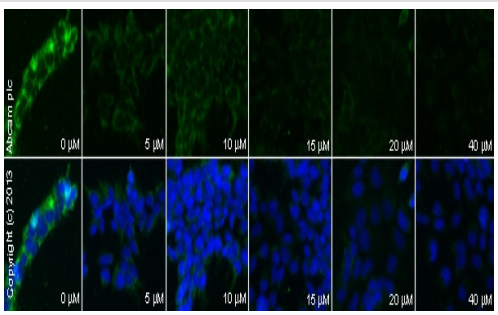


Dot Blot - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

Dot blot analysis of eIF4E (pS209) peptide (Lane 1) and eIF4E non-phospho peptide (Lane 2) labelling eIF4E (pS209) with purified ab76256 at a dilution of 1/1000. **ab97051** (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

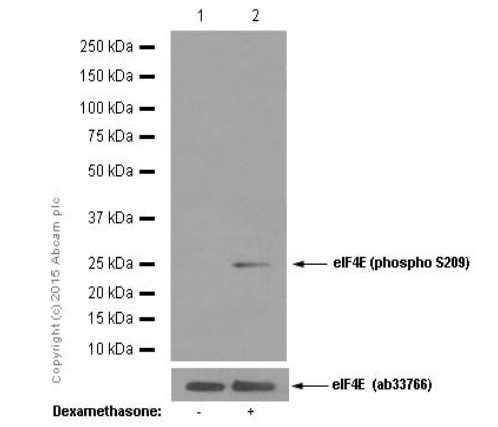
Exposure time: 3 minutes.



Immunocytochemistry/ Immunofluorescence - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

Immunocytochemistry/Immunofluorescence analysis of serum starved HEK293 cells treated with CGP 57380 **ab120365**) labelling eIF4E (phospho S209) with unpurified **ab32124** at 1/100. Decrease in eIF4E (phospho S209) expression correlates with increased concentration of CGP 57380, as described in literature.

The cells were incubated at 37°C for 1h in media containing different concentrations of **ab120365** (CGP 57380) in DMSO, fixed with 100% methanol for 5 minutes at -20°C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with unpurified ab76256 was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (**ab96899**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Western blot - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

All lanes : Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/50000 dilution (purified)

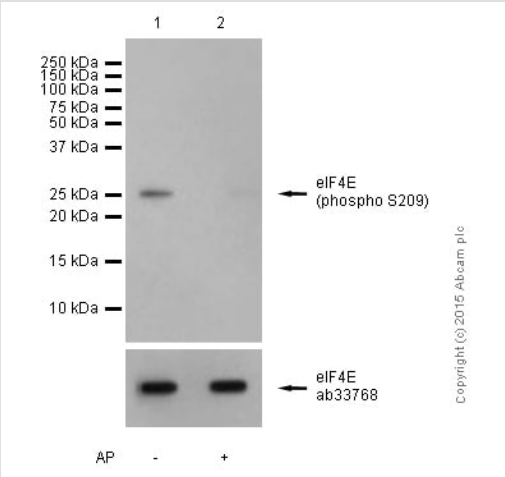
Lane 1 : Untreated HEK293 cell lysate
Lane 2 : HEK293 treated with 10mM Dexamethasone 1 hour lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 25 kDa

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

All lanes : Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/50000 dilution (purified)

Lane 1 : Untreated 293 cell lysate
Lane 2 : 293 cell lysate treated with alkaline phosphatase

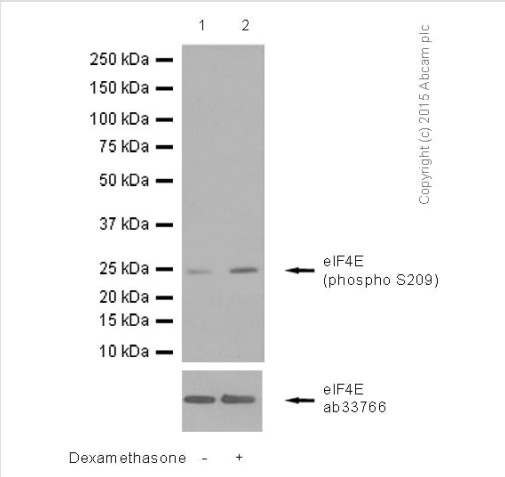
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 25 kDa

Exposure time: 1 minute

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

All lanes : Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/100000 dilution (purified)

Lane 1 : Untreated HEK293 cell lysate
Lane 2 : HEK293 cell lysate - treated with Dexamethasone

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

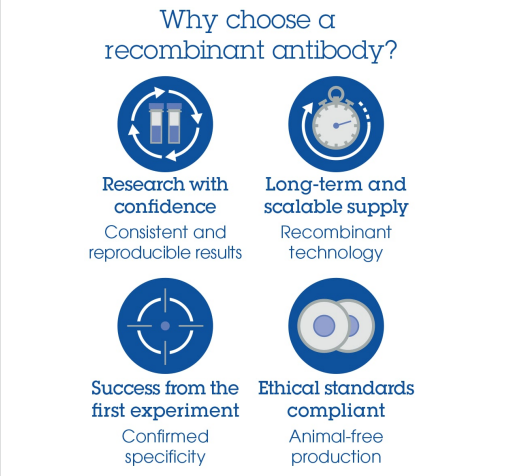
Predicted band size: 25 kDa

Exposure time:

eIF4E pS209: 15 seconds.

eIF4E: 3 minutes.

Blocking and dilution buffer: 5% NFDM/TBST.



Anti-eIF4E (phospho S209) antibody [EP2151Y]

(ab76256)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors