abcam

Product datasheet

Anti-elF4E (phospho S209) antibody [EP2151Y] ab76256



重组 RabMAb

★★★★★ 2 Abreviews 49 References 11 图像

概述

产品名称 Anti-eIF4E (phospho S209)抗体[EP2151Y]

描述 兔单克隆抗体[EP2151Y] to eIF4E (phospho S209)

宿主 Rabbit

经测试应用 适用于: ICC/IF, WB, IP, IHC-P, Dot blot

种属反应性 与反应: Mouse, Rat, Human, Pig

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 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.

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 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.

Stable for 12 months at -20°C.

pH: 7.20 存储溶液

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

纯度 Protein A purified

克隆 单克隆 克隆编号 EP2151Y

同种型 ΙgG

The Abpromise guarantee Abpromise™承诺保证使用ab76256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
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.

靶标

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 $\,$

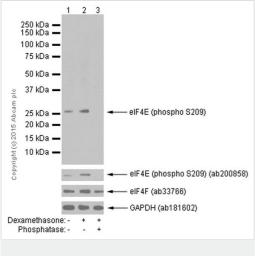
complex this subunit mediates the binding to the mRNA cap.

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



Western blot - Anti-elF4E (phospho S209) antibody [EP2151Y] (ab76256)

All lanes : Anti-elF4E (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

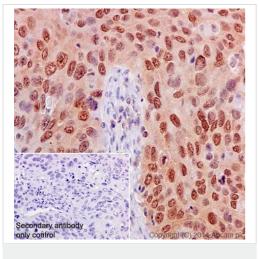
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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

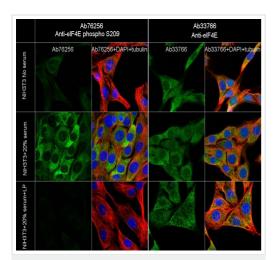
Exposure time: 30 seconds

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

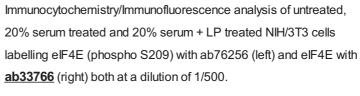
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labelling elF4E with purified ab76256 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a goat antirabbit lgG H&L (HRP) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-elF4E (phospho S209) antibody [EP2151Y] (ab76256)



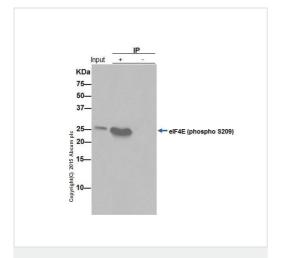
Immunocytochemistry/ Immunofluorescence - AntielF4E (phospho S209) antibody [EP2151Y] (ab76256)



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

<u>ab33766</u> 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.

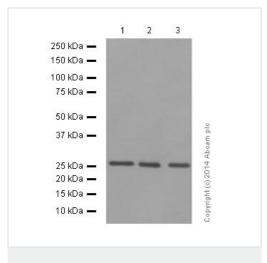


Immunoprecipitation - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

ab76256 (purified) at 1/40 immunoprecipitating elF4E (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 lgG (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-elF4E (phospho S209) antibody [EP2151Y] (ab76256)

All lanes : Anti-elF4E (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

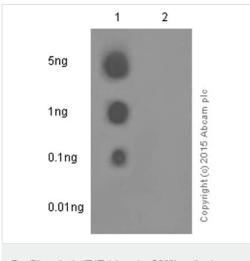
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/1000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 25 kDa

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

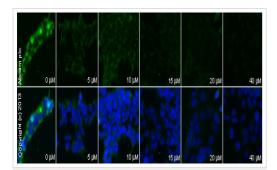


Dot Blot - Anti-elF4E (phospho S209) antibody [EP2151Y] (ab76256)

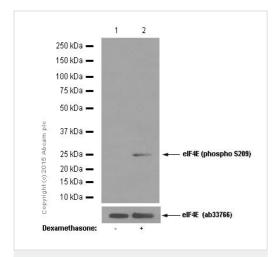
Dot blot analysis of elF4E (pS209) peptide (Lane 1) and elF4E non-phospho peptide (Lane 2) labelling elF4E (pS209) with purified ab76256 at a dilution of 1/1000. <u>ab97051</u> (Peroxidase conjugated goat anti-rabbit lgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Immunocytochemistry/ Immunofluorescence - AntielF4E (phospho S209) antibody [EP2151Y] (ab76256)



Western blot - Anti-elF4E (phospho S209) antibody [EP2151Y] (ab76256)

Immunocytochemistry/Immunofluorescence analysis of serum starved HEK293 cells treated with CGP 57380 ab120365) labelling elF4E (phospho S209) with unpurified ab32124 at 1/100. Decrease in elF4E (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.

All lanes : Anti-elF4E (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

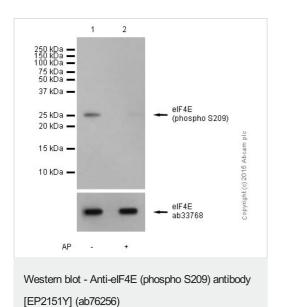
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/10000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 25 kDa

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



All lanes : Anti-elF4E (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) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 25 kDa

Exposure time: 1 minute

Blocking and dilution buffer: 5% NFDM/TBST.

1 2 Copyright (c) 2015 Abcam plc 250 kDa -150 kDa -100 kDa -75 kDa --50 kDa 🕳 37 kDa 🕳 eIF4E 25 kDa -(phospho S209) 20 kDa 🕳 15 kDa -10 kDa eIF4E ab33766 Dexamethasone -

Western blot - Anti-elF4E (phospho S209) antibody [EP2151Y] (ab76256) **All lanes :** Anti-elF4E (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) (<u>ab97051</u>) at 1/10000 dilution

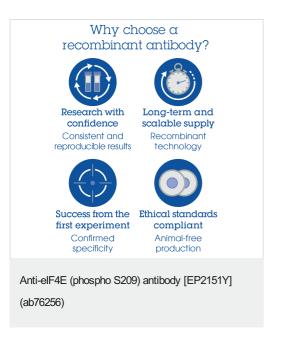
Predicted band size: 25 kDa

Exposure time:

elF4E pS209: 15 seconds.

elF4E: 3 minutes.

Blocking and dilution buffer: 5% NFDM/TBST.



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