abcam

Product datasheet

Anti-elF4E (phospho S209) antibody ab4774

1 References 画像数 3

製品の概要

製品名 Anti-elF4E (phospho S209) antibody

製品の詳細 Rabbit polyclonal to elF4E (phospho S209)

由来種 Rabbit

特異性 This phosphorylation site specific antibody is selective for eIF-4E containing a phosphate on

serine 209.

アプリケーション 適用あり: ICC/IF, WB **種交差性** 交差種: Rat, Human

交差が予測される動物種: Mouse, Rabbit, a wide range of other species

A

免疫原 Synthetic peptide corresponding to Human eIF4E (phospho S209).

ポジティブ・コントロール WB: HeLa, HEK293, L6 cell lysate ICC/IF: U-87 MG cells

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

製品の特性

特記事項

製品の状態 Liquid

保存方法 Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw

cycles.

バッファー pH: 7.30

Preservative: 0.05% Sodium azide

Constituents: PBS, 50% Glycerol (glycerin, glycerine), 1% BSA

精製度 Immunogen affinity purified

特記事項(精製) Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has

been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated eIF-4E. The final product is generated by affinity chromatography using an eIF-4E-derived peptide that is

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phosphorylated at serine 209.

ポリ/モノ ポリクローナル

アイソタイプ IgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab4774の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ICC/IF		1/250.
WB		1/1000. Detects a band of approximately 25 kDa (predicted molecular weight: 25 kDa).

ターゲット情報

機能 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

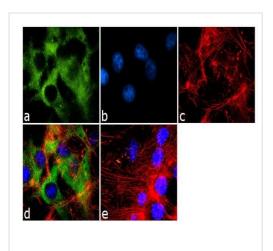
complex this subunit mediates the binding to the mRNA cap.

配列類似性 Belongs to the eukaryotic initiation factor 4E family.

翻訳後修飾 Phosphorylation increases the ability of the protein to bind to mRNA caps and to form the eIF4F

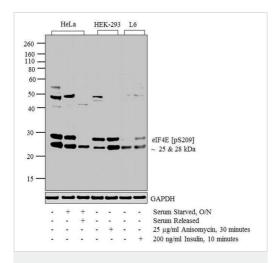
complex.

画像



Immunocytochemistry/ Immunofluorescence - AntieIF4E (phospho S209) antibody (ab4774) Immunofluorescence analysis using 70% confluent log phase U-87 MG cells labeled for Phospho-elF4E pSer209 using ab4774. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with ab4774 at 1:250 dilution in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Rabbit lgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate at a dilution of 1:2000 for 45 minutes at room temperature (Panel A: green). Nuclei (Panel B: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI. F-actin (Panel C: red) was stained with Rhodamine Phalloidin (1:300). Panel D is a merged image showing cytoplasmic localization.

captured at 60X magnification.



Western blot - Anti-elF4E (phospho S209) antibody (ab4774)

All lanes: Anti-elF4E (phospho S209) antibody (ab4774)

Lane 1: HeLa whole cell extracts

Lane 2: Serum starved HeLa

Lane 3: HeLa Serum Starved for overnight followed by Serum

Released

Lane 4: HEK-293

Lane 5: HEK-293 treated for 30 minutes with 25 µg/mL of

Anisomycin

Lane 6: L6

Lane 7: L6 treated for 10 minutes with 200 ng/mL of Insulin

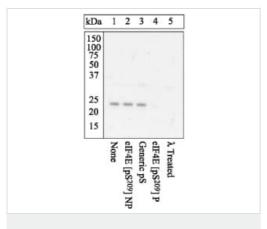
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate at 0.4 μg/ml

Predicted band size: 25 kDa

Western blot analysis was performed on HeLa, HEK-293 (human cell lines) and L6 (rat cell line) cell lysates with various treatments, blotted for elF4E (pS209) using ab4774. Two bands ~ 25 and 28 kDa band corresponding to elF4E (pS209) was observed across cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 12 % Bis-Tris gel, XCell SureLock™ Electrophoresis System and Novex® Sharp Pre-Stained Protein Standard. Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System. The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate



Western blot - Anti-elF4E (phospho S209) antibody (ab4774)

Extracts of HeLa cells were resolved by SDS-PAGE on a 4-20% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature, either left untreated (1-4) or treated with lambda (?) phosphatase (5), and then incubated with the elF4E (phospho S209) antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1,5), the nonphosphopeptide corresponding to the phosphopeptide immunogen (2), a generic phosphoserine-containing peptide (3), or the phosphopeptide immunogen (4). After washing, the membrane was incubated with goat F(ab')2 anti-rabbit lgG alkaline phosphatase and signals were detected using the Pierce SuperSignal™ method.

The data show that only the peptide corresponding to eIF4E (phospho S209) blocks the antibody signal, demonstrating the specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, verifying that the antibody i

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