abcam

Product datasheet

Anti-CREB (phospho S133) antibody [E113] ab32096

Recombinant RabMAb

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Overview

Product name	Anti-CREB (phospho S133) antibody [E113]
Description	Rabbit monoclonal [E113] to CREB (phospho S133)
Host species	Rabbit
Specificity	This antibody is specific for CREB phosphorylated on Serine 133. The immunogen of the antibody shares 94% homology with CREB (S136) and 86% homology with ATF1 (pS63). No experiment has been performed to verify the possible cross-reactivity. This antibody can't detect signal in mouse and rat brain related tissues.
Tested applications	**Suitable for:** WB, IHC-P, IP, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Chicken, Cow, Zebrafish
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A431 cell lysate; HeLa treated with anisomycin for 30 minutes ICC/IF: A431 and HeLa IHC-P: Human thyroid gland adenocarcinoma, human astrocytoma, rat cereburm and mouse cerebrum tissues. IP: HeLa treated with 25 ug/mL anisomycin for 30 minutes.
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	E113
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32096 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
WB	★★★★☆ (7)	1/5000. Predicted molecular weight: 37 kDa. **For unpurified use at 1/500.**
IHC-P	★★★★★ (3)	1/100 - 1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
IP		1/40 - 1/100.
ICC/IF	★★★★☆ (1)	1/100 - 1/250.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Target

Function This protein binds the cAMP response element (CRE), a sequence present in many viral and cellular promoters. CREB stimulates transcription on binding to the CRE. Transcription activation is enhanced by the TORC coactivators which act independently of Ser-133 phosphorylation. Implicated in synchronization of circadian rhythmicity.

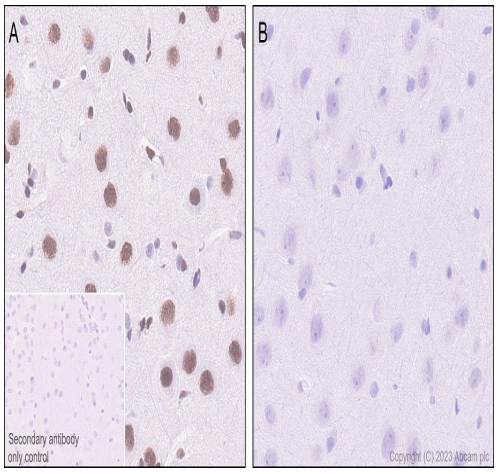
Involvement in disease Defects in CREB1 may be a cause of angiomatoid fibrous histiocytoma (AFH) [MIM:612160]. A distinct variant of malignant fibrous histiocytoma that typically occurs in children and adolescents and is manifest by nodular subcutaneous growth. Characteristic microscopic features include lobulated sheets of histiocyte-like cells intimately associated with areas of hemorrhage and cystic pseudovascular spaces, as well as a striking cuffing of inflammatory cells, mimicking a lymph node metastasis. Note=A chromosomal aberration involving CREB1 is found in a patient with angiomatoid fibrous histiocytoma. Translocation t(2;22)(q33;q12) with CREB1 generates a EWSR1/CREB1 fusion gene that is most common genetic abnormality in this tumor type.

Sequence similarities Belongs to the bZIP family.
Contains 1 bZIP domain.
Contains 1 KID (kinase-inducible) domain.

Post-translational modifications Stimulated by phosphorylation. Phosphorylation of both Ser-133 and Ser-142 in the SCN regulates the activity of CREB and participates in circadian rhythm generation. Phosphorylation of Ser-133 allows CREBBP binding (By similarity). Phosphorylated upon DNA damage, probably by ATM or ATR.

	Sumoylated by SUMO1. Sumoylation on Lys-304, but not on Lys-285, is required for nuclear localization of this protein. Sumoylation is enhanced under hypoxia, promoting nuclear localization and stabilization.
Cellular localization	Nucleus.

Images

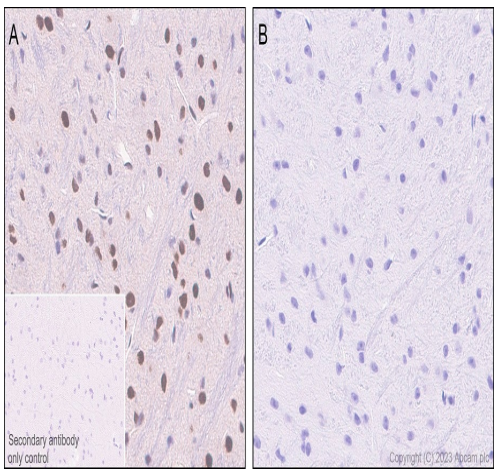


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] (ab32096)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling CREB with ab32096 at 1/2000 followed by a ready to use **ab209101**. Nuclear staining on rat cerebrum without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab32096 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use **ab209101**.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

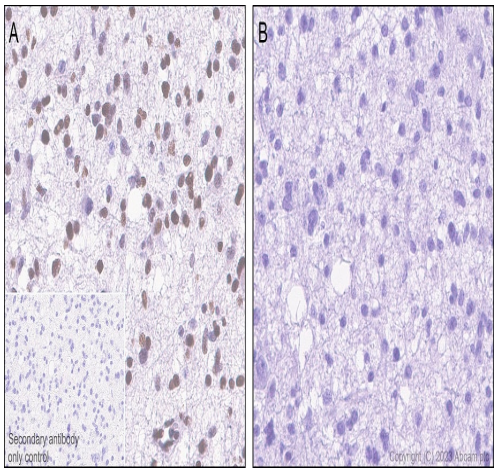


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] (ab32096)

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling CREB with ab32096 at 1/2000 followed by a ready to use **ab209101**. Nuclear staining on mouse cerebrum without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab32096 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use **ab209101**.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

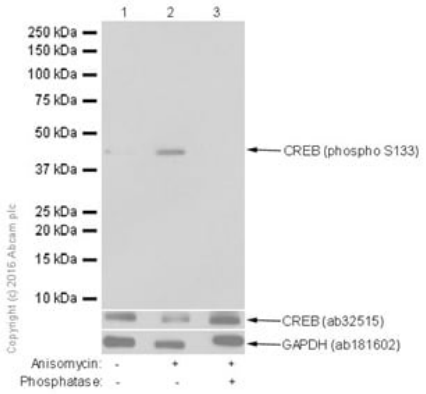


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] (ab32096)

Immunohistochemical analysis of paraffin-embedded Human astrocytoma tissue labeling CREB with ab32096 at 1/2000 followed by a ready to use **ab209101**. Nuclear staining on human astrocytoma without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab32096 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use **ab209101**.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Western blot - Anti-CREB (phospho S133) antibody [E113] (ab32096)

All lanes : Anti-CREB (phospho S133) antibody [E113] (ab32096) at 1/500 dilution

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysates
Lane 2 : HeLa whole cell lysates treated with anisomycin at 25ug/ml for 30 minutes
Lane 3 : HeLa whole cell lysates treated with anisomycin at 25ug/ml for 30 minutes, then the membrane was incubated with phosphatase

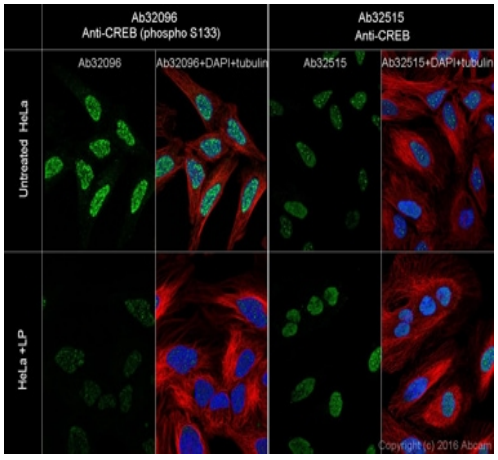
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 37 kDa
Observed band size: 40 kDa

Exposure time: 15 seconds

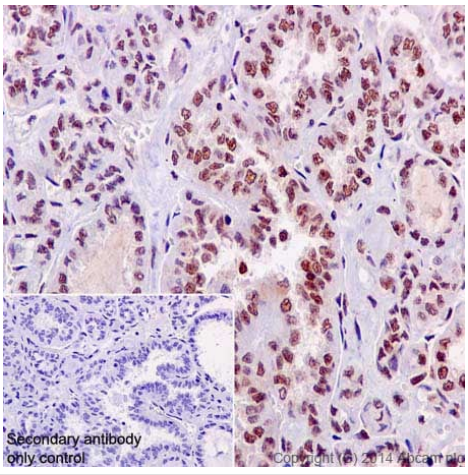
Blocking and diluting buffer and concentration: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-CREB (phospho S133) antibody [E113] (ab32096)

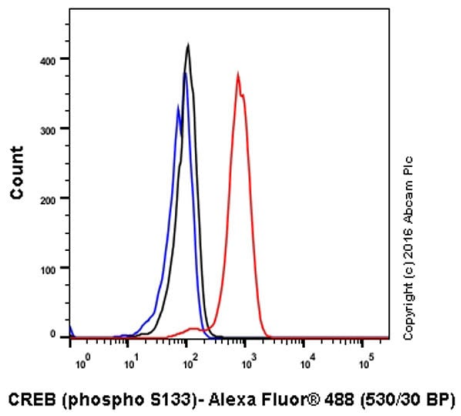
Immunocytochemistry/Immunofluorescence analysis of LP treated and untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) labelling CREB with purified ab32096 at 1/200. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain at a dilution of 1/200.

Confocal image showing nuclear staining on HeLa cells .The signal decreased after Lambda Protein Phosphatase treatment (31Ⅰ,2h).



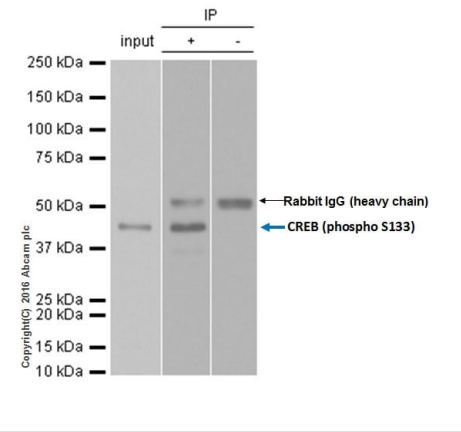
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] (ab32096)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue labelling CREB with purified ab32096 at 1/100. 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.



Flow Cytometry (Intracellular) - Anti-CREB (phospho S133) antibody [E113] (ab32096)

Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labeling CREB with purified ab32096 at 1/70 dilution (10µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Immunoprecipitation - Anti-CREB (phospho S133) antibody [E113] (ab32096)

ab32096 at 1/100 dilution immunoprecipitating CREB (phospho S133) in HeLa (human cervix adenocarcinoma) treated with 25ug/mL anisomycin for 30 minutes, whole cell lysate, observed at 40 kDa (lanes 1 and 2).

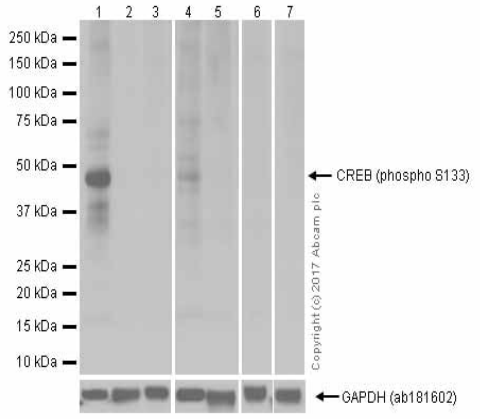
Lane 1 (input): HeLa treated with 25ug/mL anisomycin for 30 minutes. Whole cell lysate, 10µg.

Lane 2 (+): ab32096 + HeLa treated with 25ug/mL anisomycin for 30 minutes. Whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32096 in treated with 25ug/mL anisomycin for 30 minutes. Whole cell lysate

For western blotting, ab32096 at 1/200 dilution followed by **ab131366** VeriBlot for IP Detection Reagent (HRP) at 1/1000 for detection.

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



Western blot - Anti-CREB (phospho S133) antibody [E113] (ab32096)

All lanes : Anti-CREB (phospho S133) antibody [E113] (ab32096) at 1/200 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with 250ng/ml anisomycin for 30 minutes whole cell lysates
Lane 2 : Mouse brain lysates
Lane 3 : Rat brain lysates
Lane 4 : Rat cerebellum lysate
Lane 5 : Mouse hippocampus lysate
Lane 6 : Rat hippocampus lysate
Lane 7 : Rat cerebral cortex lysate

Lysates/proteins at 15 µg per lane.

Secondary

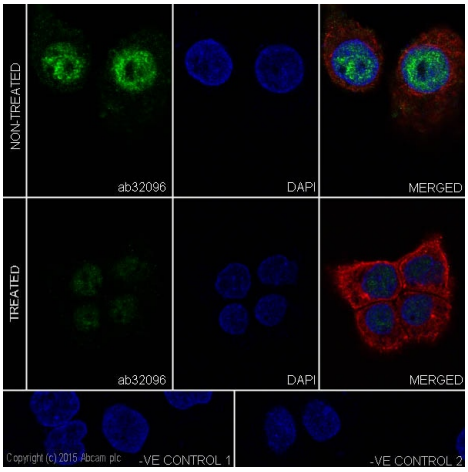
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 37 kDa

Exposure time: 3 seconds

Blocking and diluting buffer: 5% NFDM/TBST

This antibody can`t detect signal in mouse and rat brain related tissues.

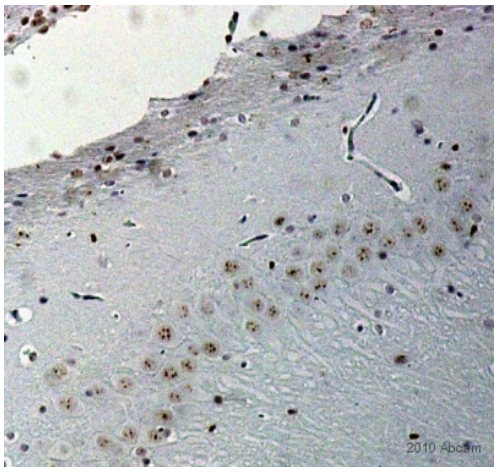


Immunocytochemistry/ Immunofluorescence - Anti-CREB (phospho S133) antibody [E113] (ab32096)

Immunocytochemistry/Immunofluorescence analysis of A431(human epidermoid carcinoma) cells +/- AP 37▌ 1h labelling CREB with purified ab32096 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) 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.

Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

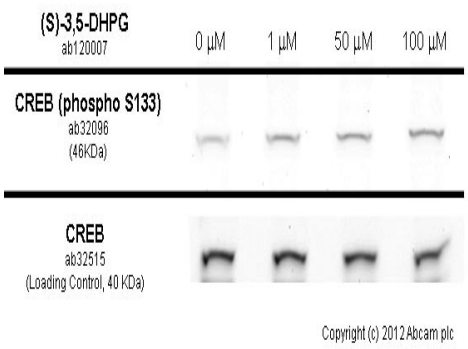
Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] (ab32096)

This image is courtesy of an Abreivew submitted by Akiko Shingo.

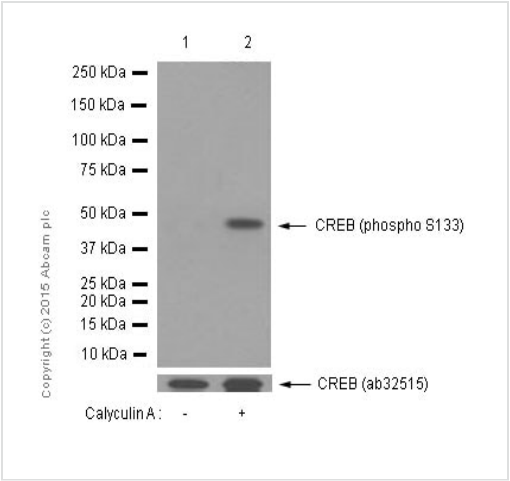
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat hippocampus tissue labelling CREB with unpurified ab32096 at 1/100 dilution. Sections were subjected to antigen retrieval by autoclave prior to blocking with 8% milk for 30 minutes at 37°C. The primary antibody was diluted 1/100 with DAKO antibody diluent and incubated with the sample for 18 hours at 4°C. An LSAB-labeled Streptavidin-Biotin conjugated Goat polyclonal antibody was used undiluted as the secondary antibody.



Western blot - Anti-CREB (phospho S133) antibody [E113] (ab32096)

SK-N-SH cells were incubated at 37&degC for 30 minutes with vehicle control (0 &microM) and different concentrations of (S)-3,5-DHPG (**ab120007**). Increased expression of CREB (phospho S133) in SK-N-SH cells correlates with an increase in (S)-3,5-DHPGconcentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 20&microg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 3% milk before being incubated with unpurified ab32096 at 1/500 dilution and **ab32515** at 1 &microg/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (**ab97051**) at 1/10000 dilution and visualised using ECL development solution.



Western blot - Anti-CREB (phospho S133) antibody [E113] (ab32096)

Lane 1 : Anti-CREB (phospho S133) antibody [E113] (ab32096) at 1/5000 dilution (purified)
Lane 2 : purified at 1/5000 dilution

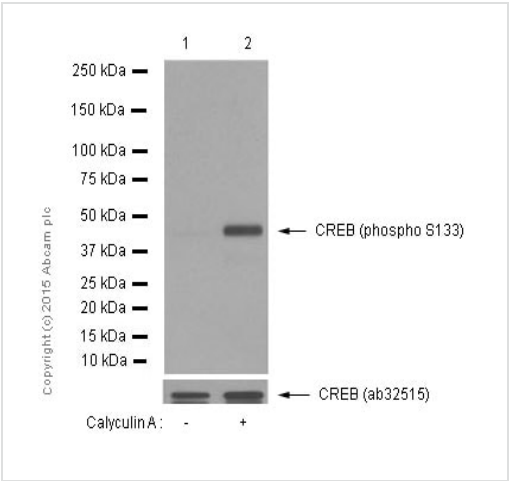
Lane 1 : Untreated C6 cell lysate
Lane 2 : C6 treated with Calyculin A cell lysate

Lysates/proteins at 10 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: 37 kDa

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



Western blot - Anti-CREB (phospho S133) antibody [E113] (ab32096)

All lanes : Anti-CREB (phospho S133) antibody [E113] (ab32096) at 1/5000 dilution (purified)

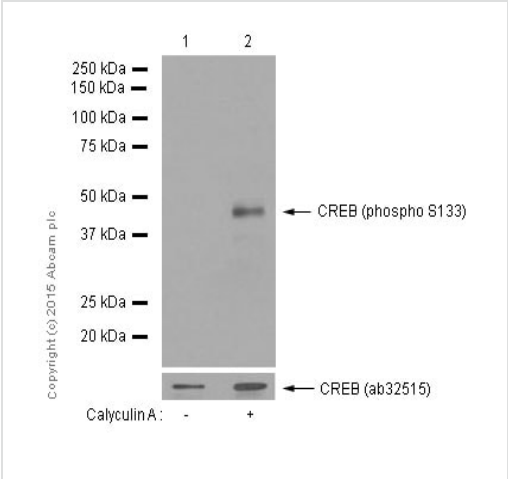
Lane 1 : Untreated NIH/3T3 cell lysate
Lane 2 : NIH/3T3 treated with Calyculin A

Lysates/proteins at 10 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: 37 kDa

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



Western blot - Anti-CREB (phospho S133) antibody [E113] (ab32096)

All lanes : Anti-CREB (phospho S133) antibody [E113] (ab32096) at 1/5000 dilution (purified)

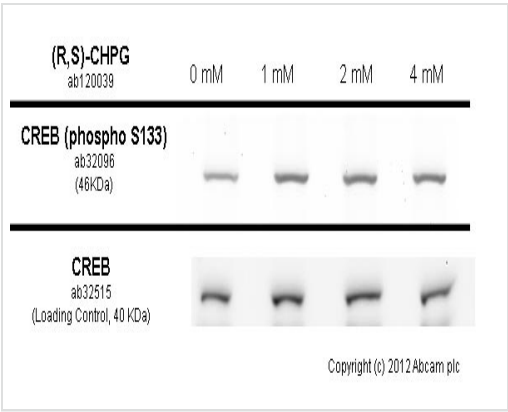
Lane 1 : Untreated HeLa cell lysate
Lane 2 : HeLa treated with Calyculin A cell lysate

Lysates/proteins at 10 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: 37 kDa

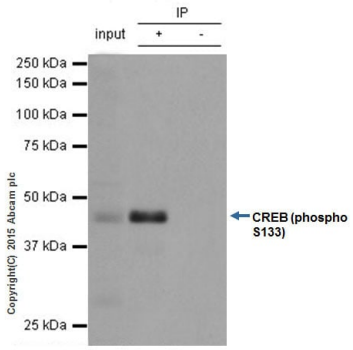
Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-CREB (phospho S133) antibody [E113] (ab32096)

SK-N-SH cells were incubated at 37&degC for 30 minutes with vehicle control (0 &microM) and different concentrations of (R,S)-CHPG (**ab120039**). Increased expression of CREB (phospho S133) in SK-N-SH cells correlates with an increase in (R,S)-CHPG concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 20&microg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 3% milk before being incubated with unpurified ab32096 at 1/500 dilution and **ab32515** at 1 &microg/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (**ab97051**) at 1/10000 dilution and visualised using ECL development solution.

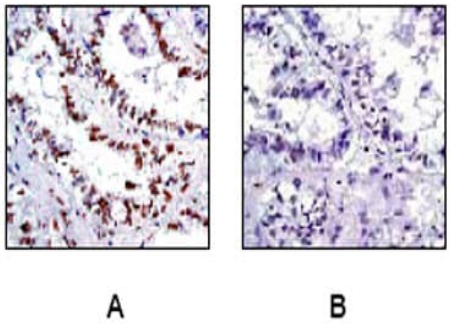


Immunoprecipitation - Anti-CREB (phospho S133) antibody [E113] (ab32096)

ab32096 (purified) at 1/50 immunoprecipitating CREB (phospho S133) in HeLa 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.

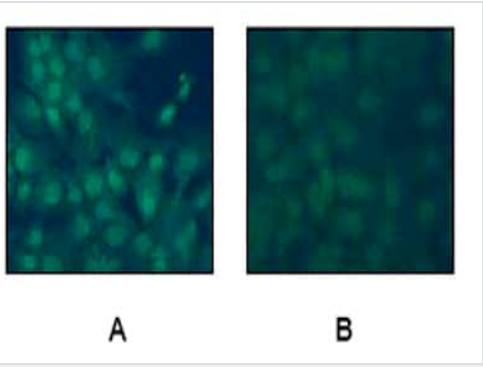
Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] (ab32096)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid gland adenocarcinoma tissue labelling CREB with unpurified ab32096 at 1/250 dilution.

Panel A: Cells are untreated.
Panel B: Cells are treated with Phosphatase.



Immunocytochemistry/ Immunofluorescence - Anti-CREB (phospho S133) antibody [E113] (ab32096)

Immunocytochemistry/Immunofluorescence analysis of A431 cells labelling CREB with unpurified ab32096 at 1/250.

Panel A: Cells are untreated.

Panel B: Cells are treated with Phosphatase.

Anti-CREB (phospho S133) antibody [E113] (ab32096)

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