abcam

Product datasheet

Anti-CREB (phospho S129 + S133) antibody ab10564

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Overview

Product name	Anti-CREB (phospho S129 + S133) antibody
Description	Rabbit polyclonal to CREB (phospho S129 + S133)
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide corresponding to Human CREB (phospho S129 + S133).
Positive control	WB: NIH3T3 cell extract with various treatments; A549 cell extract with various treatments; NIH3T3, A549, SK-N-SH, MCF7, and MDA-MB-231 untreated, EGF-treated or PDGF-treated cell lysates. ICC/IF: 70% confluent log phase HeLa cells.
General notes	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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.3 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol, 0.1% BSA PBS is Ca2+ and Mg2+ free
Purity	Immunogen affinity purified
Purification notes	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a spanning peptide corresponding to the cleavage-site to remove antibody that is reactive with non-phosphorylated CREB. The final product is generated

	by affinity chromatography using a CREB-derived peptide that is phosphorylated at serines 129 and 133.
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab10564 in the following tested applications.

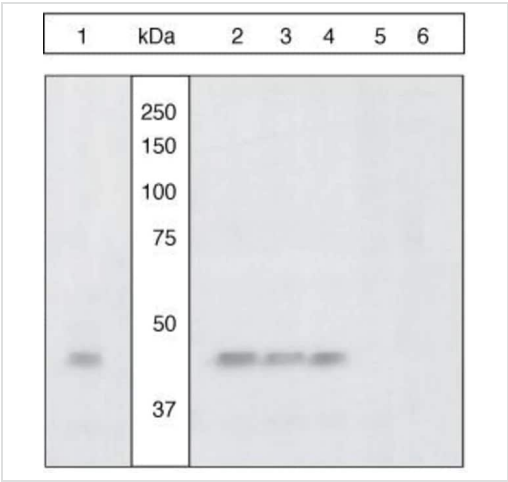
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/100 - 1/500.
WB	★★☆☆☆ **(2)**	1/1000. Detects a band of approximately 43 kDa (predicted molecular weight: 37 kDa).

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



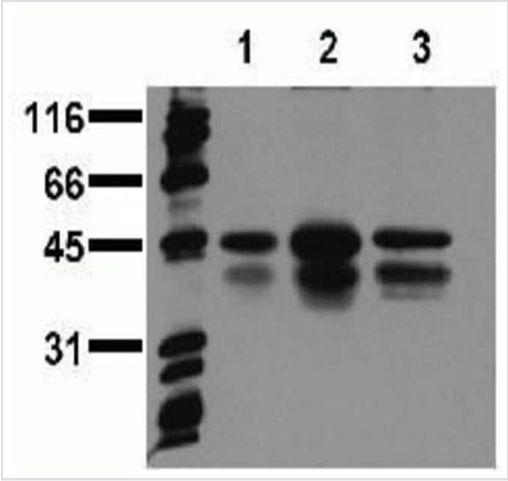
Western blot - Anti-CREB (phospho S129 + S133) antibody (ab10564)

All lanes : Anti-CREB (phospho S129 + S133) antibody (ab10564) at 1/1000 dilution

Lane 1 : NIH3T3 cell extract
Lane 2 : NIH3T3 treated with 50 ng/mL PDGF for 15 minutes cell extract
Lane 3 : NIH3T3 treated with 50 ng/mL PDGF for 15 minutes cell extract, incubated with the non-phosphopeptide corresponding to the phosphopeptide immunogen
Lane 4 : NIH3T3 treated with 50 ng/mL PDGF for 15 minutes cell extract, incubated with a generic phosphoserine-containing peptide
Lane 5 : NIH3T3 treated with 50 ng/mL PDGF for 15 minutes cell extract, incubated with the phosphopeptide immunogen
Lane 6 : NIH3T3 treated with 50 ng/mL PDGF for 15 minutes cell extract, treated with lambda phosphatase

Predicted band size: 37 kDa

The membrane was incubated with goat F(ab')2 anti-rabbit IgG HRP conjugate and signals were detected using the Pierce SuperSignal™ method.



Western blot - Anti-CREB (phospho S129 + S133) antibody (ab10564)

All lanes : Anti-CREB (phospho S129 + S133) antibody (ab10564) at 0.5 µg/ml

Lane 1 : Human A549 whole cell extract
Lane 2 : Human A549 whole cell extract stimulated with EGF
Lane 3 : Human A549 whole cell extract treated with pervanadate

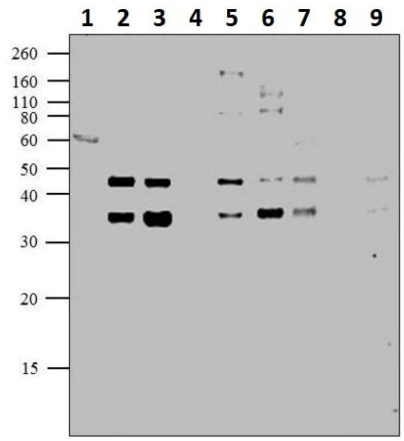
Lysates/proteins at 20000 cells per lane.

Secondary
All lanes : Anti-mouse, HRP-conjugated

Developed using the ECL technique.

Predicted band size: 37 kDa

Exposure time: 30 seconds



Western blot - Anti-CREB (phospho S129 + S133) antibody (ab10564)

All lanes : Anti-CREB (phospho S129 + S133) antibody (ab10564) at 1/1000 dilution

Lane 1 : NIH/3T3 cell lysate
Lane 2 : NIH/3T3 treated for 10 minutes with 50 ng/mL of PDGF cell lysate
Lane 3 : NIH/3T3 treated for 10 minutes with 200 ng/mL of EGF cell lysate
Lane 4 : A549 cell lysate
Lane 5 : A549 treated for 10 minutes with 200 ng/mL of EGF cell lysate
Lane 6 : A431 cell lysate
Lane 7 : SK-N-SH cell lysate
Lane 8 : MCF7 cell lysate
Lane 9 : MDA-MB-231 cell lysate

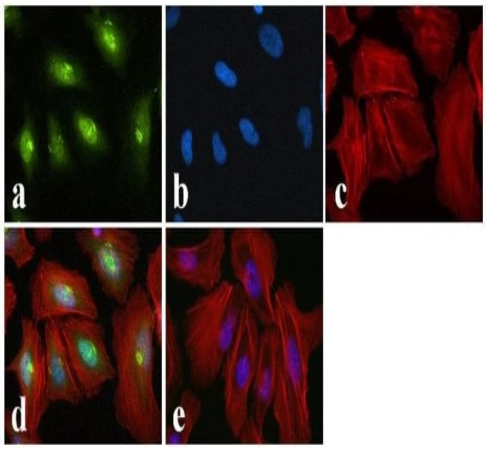
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG - HRP at 1/5000 dilution

Developed using the ECL technique.

Predicted band size: 37 kDa
Observed band size: 35,43 kDa

Detection: Chemiluminescence.



Immunocytochemistry/ Immunofluorescence - Anti-CREB (phospho S129 + S133) antibody (ab10564)

Immunofluorescent analysis of CREB (phospho S129/pS133) was done on 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton X-100 for 10 minutes, and blocked with 5% BSA for 1 hour at room temperature. The cells were labeled with CREB (phospho S129/pS133) Rabbit polyclonal Antibody (ab10564) at 2 µg/mL in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor 488 Goat Anti-Rabbit IgG Secondary Antibody at a dilution of 1/400 for 30 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 Alexa Fluor 594 Phalloidin. Panel d is a merged image showing nuclear localization. Panel e shows no primary antibody control. The images were captured at 20X

magnification.

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