# abcam

# Product datasheet

# Anti-CREB antibody [E306] - BSA and Azide free ab221216



Recombinant

RabMAb

# 12 References 4 Images

#### Overview

Product name Anti-CREB antibody [E306] - BSA and Azide free

**Description** Rabbit monoclonal [E306] to CREB - BSA and Azide free

Host species Rabbit

**Specificity** This antibody recognizes CREB, and can also recognize the splice isoform of CREB.

Tested applications Suitable for: WB, IHC-P

**Species reactivity** Reacts with: Mouse, Rat, Human

Predicted to work with: Cow

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control ICC/IF: PC 12 cells. WB: A431 cell lysate. IHC-P: Prostatic carcinoma tissue.

**General notes** ab221216 is the carrier-free version of **ab32515**.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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

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## **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

**Clonality** Monoclonal

Clone number E306
Isotype IgG

## **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab221216 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		Use at an assay dependent concentration. Detects a band of approximately 40 kDa (predicted molecular weight: 37 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

# **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

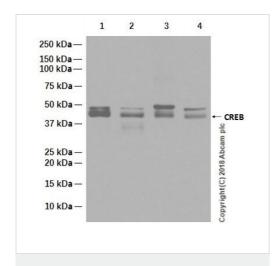
Stimulated by phosphorylation. Phosphorylation of both Ser-133 and Ser-142 in the SCN regulates the activity of CREB and participates in circadian rhythm generation. Phosphorylation of Ser-133 allows CREBBP binding (By similarity). Phosphorylated upon DNA damage, probably by ATM or ATR.

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 antibody [E306] - BSA and Azide free (ab221216)

All lanes: Anti-CREB antibody [E306] (ab32515) at 0.03 µg/ml

**Lane 1 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20  $\mu g$ 

Lane 2 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate at 20  $\mu g$ 

Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate at 20 µg

Lane 4: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

# **Secondary**

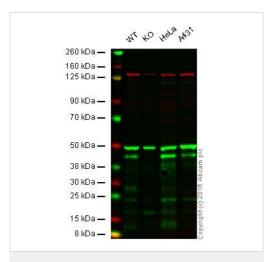
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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

Exposure time: 15 seconds

This data was developed using <u>ab32515</u>, the same antibody clone in a different buffer formulation

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-CREB antibody [E306] - BSA and Azide free (ab221216)

This data was developed using <u>ab32515</u>, the same antibody clone in a different buffer formulation:

Lane 1: Wild-type HAP1 cell lysate (20 µg)

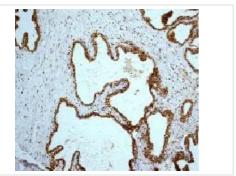
Lane 2: CREB knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: A431 cell lysate(20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab32515</u> observed at 44 kDa. Red - loading control, <u>ab18058</u>, observed at 214 kDa.

ab32515 was shown to recognize CREB in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when CREB knockout samples examined. Wild-type and knockout samples were subjected to SDS-PAGE. ab32515 and ab18058 (loading control to Vinculin) diluted to 1/500 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

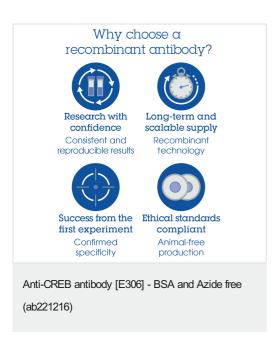


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CREB antibody [E306] - BSA and Azide free (ab221216)

Ab32515, at a 1/500 dilution, staining CREB in paraffin embedded human prostatic carcinoma tissue sections by Immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32515).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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