abcam

Product datasheet

Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free ab220798

Recombinant RabMAb

15 References 14 Images

Overview

Product name	Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free
Description	Rabbit monoclonal [E113] to CREB (phospho S133) - BSA and Azide free
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:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
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. IF: A431 cells. IHC-P: Human thyroid gland adenocarcinoma, human astrocytoma, rat cereburm and mouse cerebrum tissues. IP: HeLa treated with 25ug/mL anisomycin for 30 minutes.
General notes	ab220798 is the carrier-free version of **ab32096**. Our **carrier-free** 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 **conjugation kits** 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® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® 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 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. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E113
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab220798 in the following tested applications.

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

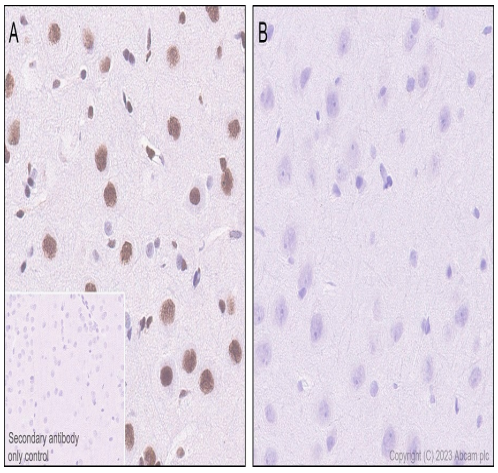
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 37 kDa.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		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



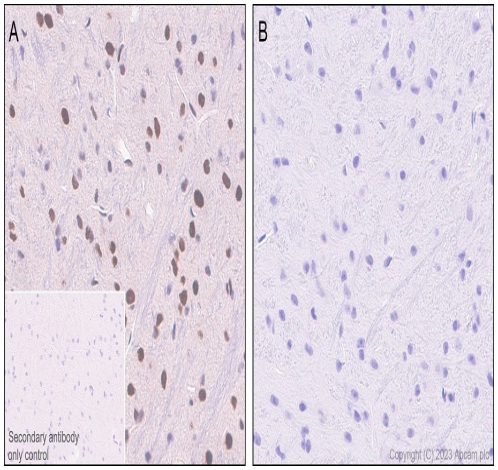
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

This data was developed using **ab32096**, the same antibody clone in a different buffer formulation.

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.



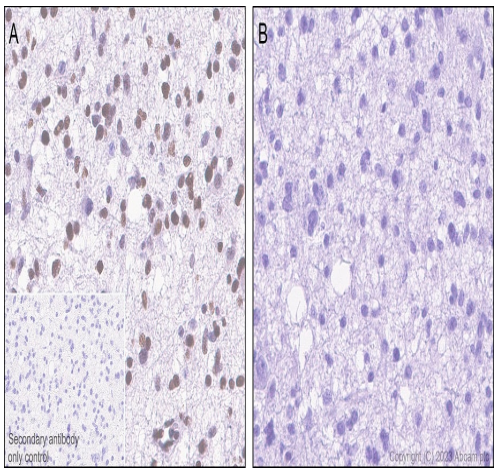
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

This data was developed using **ab32096**, the same antibody clone in a different buffer formulation.

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.



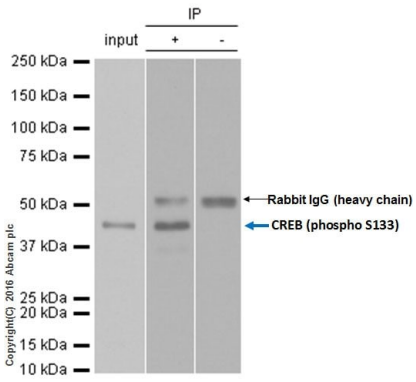
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

This data was developed using **ab32096**, the same antibody clone in a different buffer formulation.

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.



Immunoprecipitation - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

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.

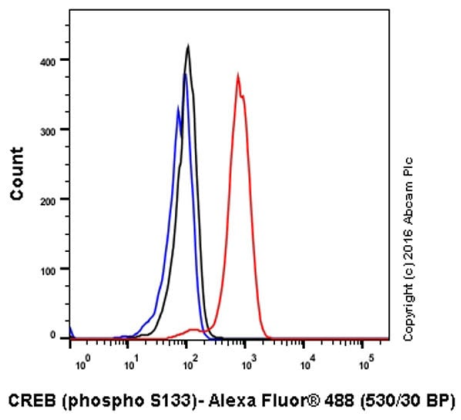
Lane 2 (+): **ab32096** + HeLa treated with 25ug/mL anisomycin for 30 minutes. Whole cell lysate.

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 (HRP) at 1/1000 as the secondary antibody.

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

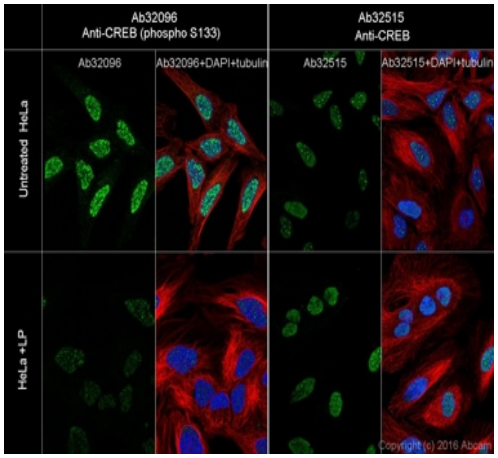
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).



Flow Cytometry (Intracellular) - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

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 Fluor® 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).

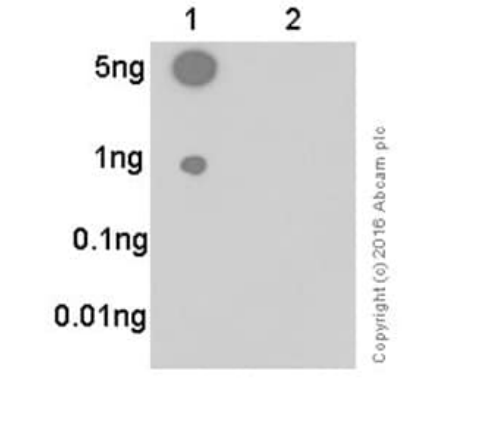


Immunocytochemistry/ Immunofluorescence - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

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 (31?,2h).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).



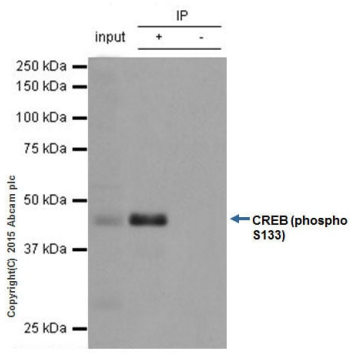
Dot Blot - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

Dot blot analysis of CREB (pS133) phospho peptide (Lane 1) and CREB non-phospho peptide (Lane 2) using **ab32096** at 1/1000 dilution followed by Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution.

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

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).



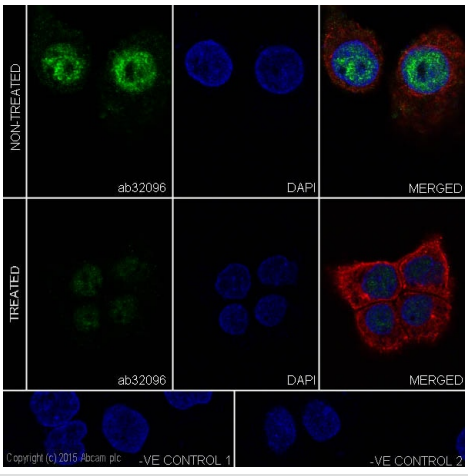
Immunoprecipitation - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

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**) was used for detection at 1/1,500 dilution. 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).



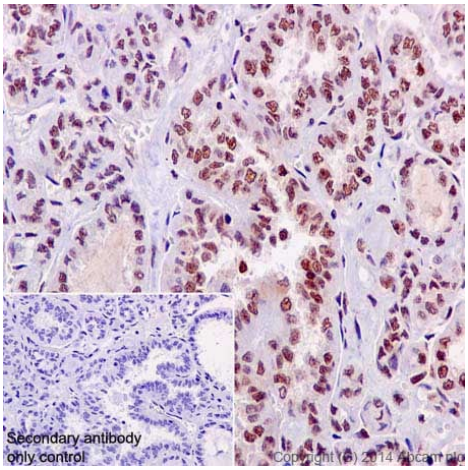
Immunocytochemistry/ Immunofluorescence - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

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).

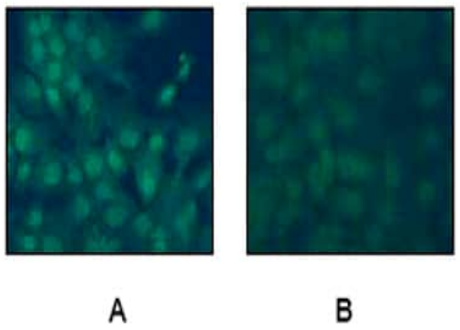
Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).



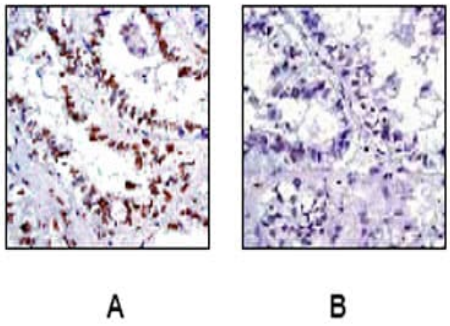
Immunocytochemistry/ Immunofluorescence - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).

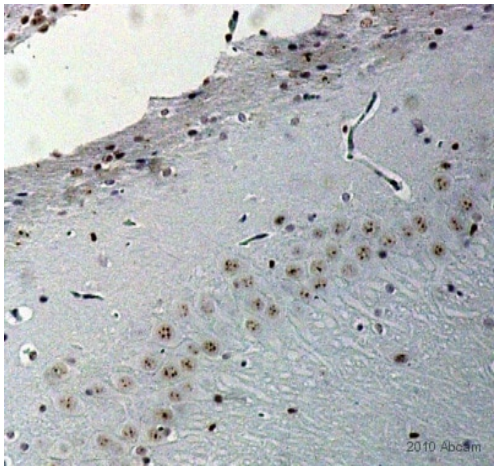


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).

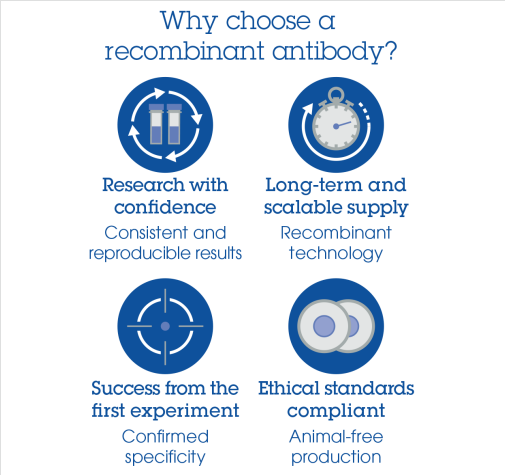


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

This image is courtesy of an Abreview 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32096**).



Anti-CREB (phospho S133) antibody [E113] - BSA and Azide free (ab220798)

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