Anti-Phospho-Creb (S133) Antibody [JB25-40] - BSA and Azide free

HA750465

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IP, FC, IF-Tissue
Molecular Wt:	Predicted band size: 35 kDa
Clone number:	JB25-40

Description: Phosphorylation-dependent transcription factor that stimulates transcription upon binding to the DNA cAMP response element (CRE), a sequence present in many viral and cellular promoters. Transcription activation is enhanced by the TORC coactivators which act independently of Ser-133 phosphorylation. Involved in different cellular processes including the synchronization of circadian rhythmicity and the differentiation of adipose cells.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser133 of human Creb.

Positive control: HeLa treated with 25μg/mL anisomycin for 30 minutes whole cell lysate, SH-SY5Y, HUVEC, human spleen tissue, mouse colon tissue, human colon carcinoma tissue, human lymph nodes tissue, mouse large intestine tissue, HeLa.

Subcellular location: Nucleus.

Database links: SwissProt: P16220 Human | Q01147 Mouse | P15337 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:100
IHC-P	1:200-1:1,000
FC	1:50-1:100
IP	Use at an assay dependent concentration.
IF-Tissue	1:200

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

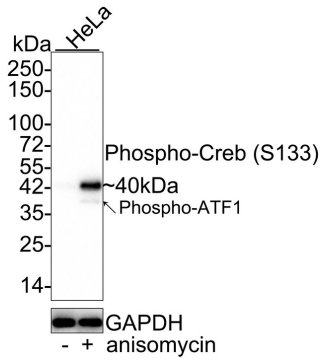

Fig1: Western blot analysis of Phospho-Creb (S133) on different lysates with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/1,000 dilution.

Lane 1: HeLa whole cell lysate
Lane 2: HeLa treated with 25µg/mL anisomycin for 30 minutes whole cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 35 kDa
Observed band size: 40 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750465) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

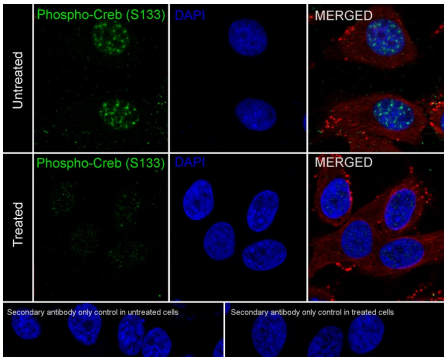

Fig2: Immunocytochemistry analysis of HeLa cells treated with or without Lambda Protein Phosphatase for 1 hour labeling Phospho-Creb (S133) with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/100 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

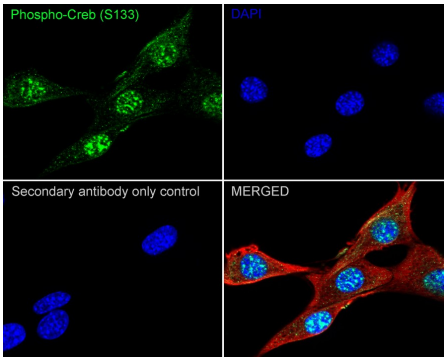

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling Phospho-Creb (S133) with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

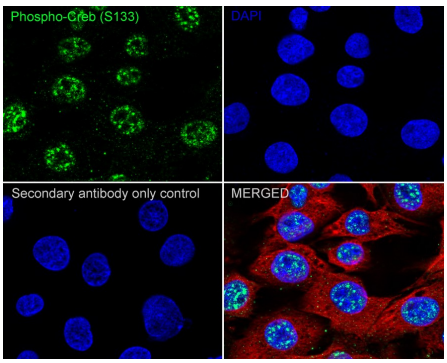

Fig4: Immunocytochemistry analysis of C6 cells labeling Phospho-Creb (S133) with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

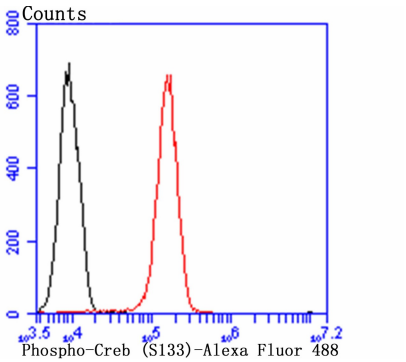

Fig5: Flow cytometric analysis of Phospho-Creb (S133) was done on HUVEC cells. The cells were fixed, permeabilized and stained with the primary antibody (HA750465, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

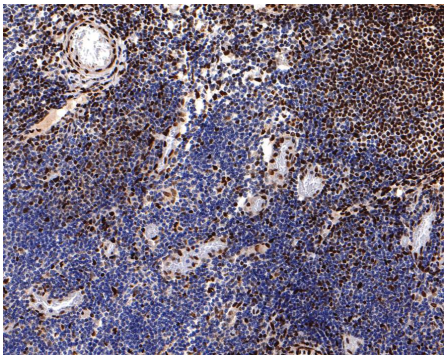

Fig6: Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750465) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

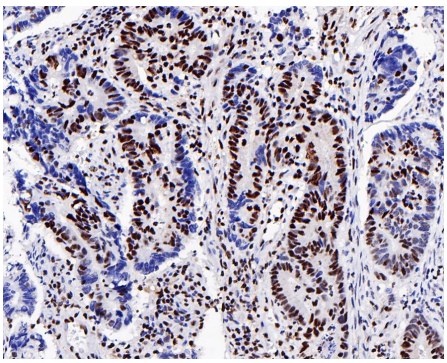

Fig7: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750465) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

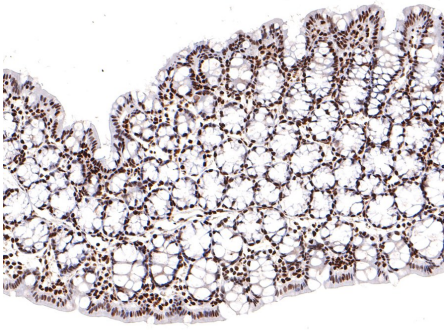

Fig8: Immunohistochemical analysis of paraffin-embedded mouse large intestine tissue with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750465) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

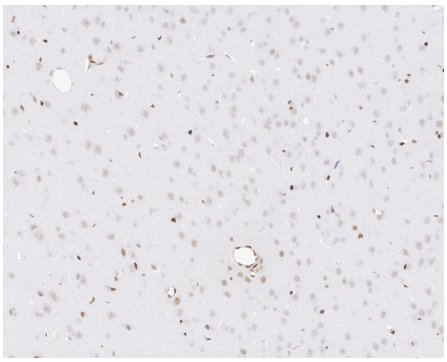

Fig9: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750465) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

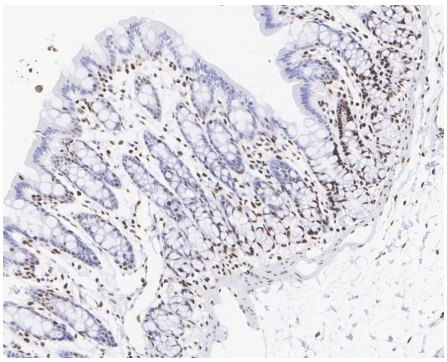

Fig10: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Phospho-Creb (S133) antibody (HA750465) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750465) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Comerford K M et al. Small ubiquitin-related modifier-1 modification mediates resolution of CREB-dependent responses to hypoxia. Proc Natl Acad Sci USA 100:986-991 (2003).
2. Kitazawa S et al. A p.D116G mutation in CREB1 leads to novel multiple malformation syndrome resembling CrebA knockout mouse. Hum Mutat 33:651-654 (2012).