

# Anti-CREB Antibody [SA04-04] - BSA and Azide free

## HA750007



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Zebrafish, Rat
<b>Applications:</b>	WB, IF-Cell, IF-Tissue, IHC-P, FC
<b>Molecular Wt:</b>	Predicted band size: 35 kDa
<b>Clone number:</b>	SA04-04

<b>Description:</b>	CREB-TF (CREB, cAMP response element-binding protein) is a cellular transcription factor. It binds to certain DNA sequences called cAMP response elements (CRE), thereby increasing or decreasing the transcription of the genes. CREB was first described in 1987 as a cAMP-responsive transcription factor regulating the somatostatin gene. Genes whose transcription is regulated by CREB include: c-fos, BDNF, tyrosine hydroxylase, numerous neuropeptides (such as somatostatin, enkephalin, VGF, corticotropin-releasing hormone), and genes involved in the mammalian circadian clock (PER1, PER2). CREB is closely related in structure and function to CREM (cAMP response element modulator) and ATF-1 (activating transcription factor-1) proteins. CREB has a well-documented role in neuronal plasticity and long-term memory formation in the brain and has been shown to be integral in the formation of spatial memory.
<b>Immunogen:</b>	Synthetic peptide within human CREB aa 250-290.
<b>Positive control:</b>	HeLa cell lysate, A431 cell lysate, U-2 OS cell lysate, HEK-293 cell lysate, COS-1 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, C6 cell lysate, PC-12 cell lysate, rat testis tissue lysate, mouse brain tissue lysate, rat brain tissue lysate, zebrafish tissue lysates, Hela, human tonsil tissue, human lung carcinoma tissue, human breast carcinoma tissue, human thyroid tissue, human stomach carcinoma tissue, human pancreas tissue, mouse colon tissue, rat brain tissue, NIH/3T3, C6, human cerebellum tissue, mouse cerebellum tissue, rat cerebellum tissue.
<b>Subcellular location:</b>	Nucleus
<b>Database links:</b>	SwissProt: P16220 Human   Q01147 Mouse   P15337 Rat
<b>Recommended Dilutions:</b>	
<b>WB</b>	1:500-1:1,000
<b>IF-Cell</b>	1:500
<b>IF-Tissue</b>	1:200
<b>IHC-P</b>	1:500-1:5,000
<b>FC</b>	1:1,000
<b>Storage Buffer:</b>	PBS (pH7.4).
<b>Storage Instruction:</b>	Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.
<b>Purity:</b>	Protein A affinity purified.

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Orders:0086-571-88062880

Technical:0086-571-89986345

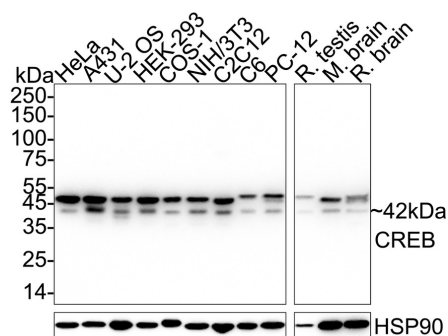
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

## Images

**Fig1:** Western blot analysis of CREB on different lysates with Rabbit anti-CREB antibody (HA750007) at 1/2,000 dilution.



Lane 1: HeLa cell lysate (20 µg/Lane)  
 Lane 2: A431 cell lysate (20 µg/Lane)  
 Lane 3: U-2 OS cell lysate (20 µg/Lane)  
 Lane 4: HEK-293 cell lysate (20 µg/Lane)  
 Lane 5: COS-1 cell lysate (20 µg/Lane)  
 Lane 6: NIH/3T3 cell lysate (20 µg/Lane)  
 Lane 7: C2C12 cell lysate (20 µg/Lane)  
 Lane 8: C6 cell lysate (20 µg/Lane)  
 Lane 9: PC-12 cell lysate (20 µg/Lane)  
 Lane 10: Rat testis tissue lysate (40 µg/Lane)  
 Lane 11: Mouse brain tissue lysate (40 µg/Lane)  
 Lane 12: Rat brain tissue lysate (40 µg/Lane)

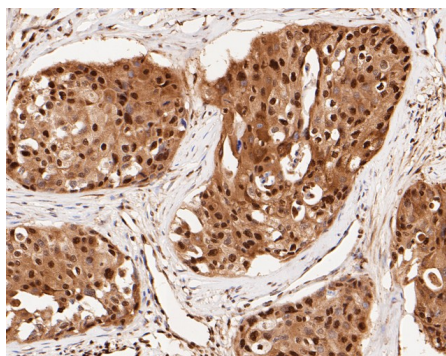
Predicted band size: 35 kDa

Observed band size: 42 kDa

Exposure time: 6 seconds; ECL: K1801;

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 (HA750007) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



**Fig2:** Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-CREB antibody.

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 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750007, 1/500) for 30 minutes 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.

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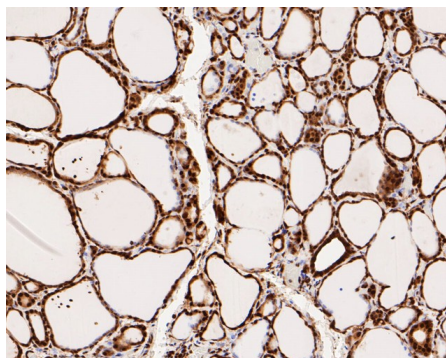
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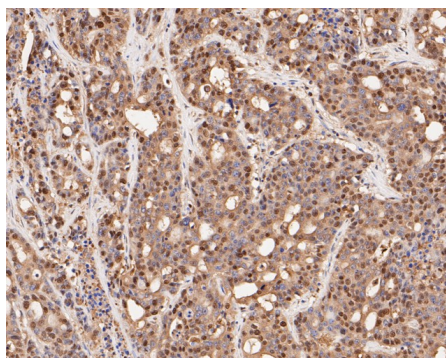
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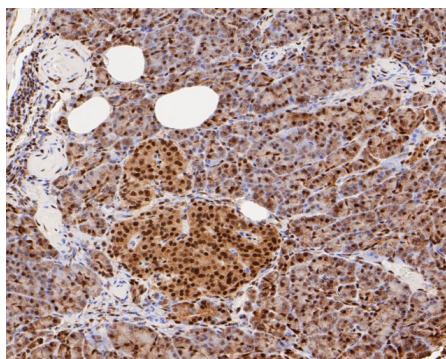
**Fig3:** Immunohistochemical analysis of paraffin-embedded human thyroid tissue using anti-CREB antibody.

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 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750007, 1/500) for 30 minutes 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.



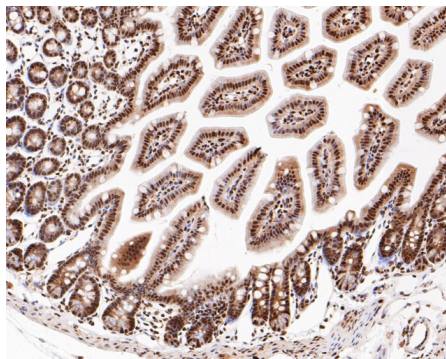
**Fig4:** Immunohistochemical analysis of paraffin-embedded human stomach carcinoma tissue using anti-CREB antibody.

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 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750007, 1/500) for 30 minutes 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.



**Fig5:** Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-CREB antibody.

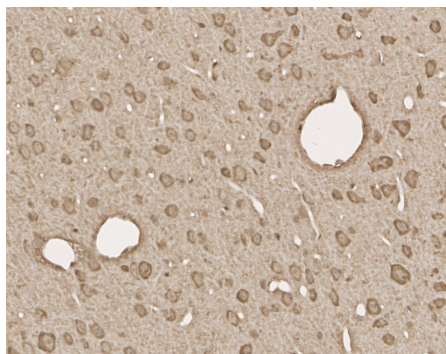
The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750007, 1/500) for 30 minutes 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.



**Fig6:** Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-CREB antibody.

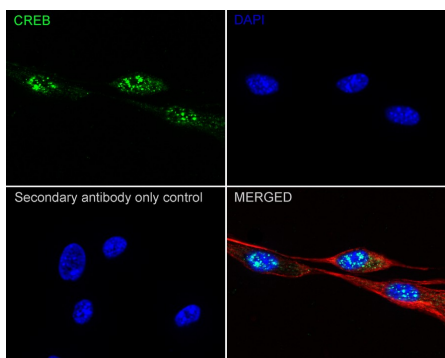
The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750007, 1/500) for 30 minutes 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 rat brain tissue with Rabbit anti-CREB antibody (HA750007) 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<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750007) 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.

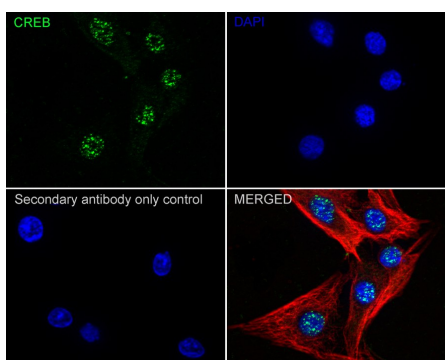


**Fig8:** Immunocytochemistry analysis of NIH/3T3 cells labeling CREB with Rabbit anti-CREB antibody (HA750007) at 1/500 dilution.

Cells were fixed in 100% precooled methanol 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-CREB antibody (HA750007) at 1/500 dilution in 1% BSA in PBST overnight at 4 °C. 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.

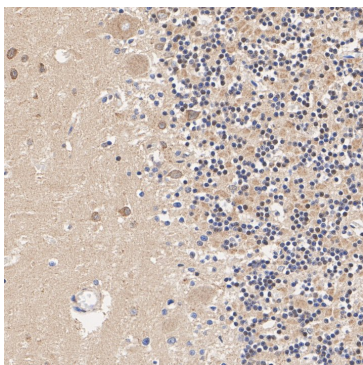
Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

**Fig9:** Immunocytochemistry analysis of C6 cells labeling CREB with Rabbit anti-CREB antibody (HA750007) at 1/500 dilution.



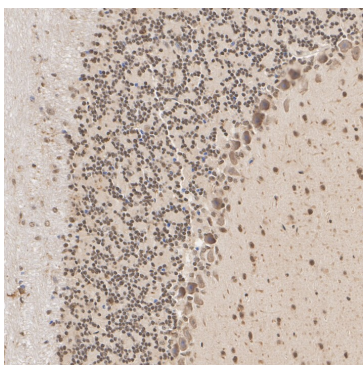
Cells were fixed in 100% precooled methanol for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CREB antibody (HA750007) at 1/500 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



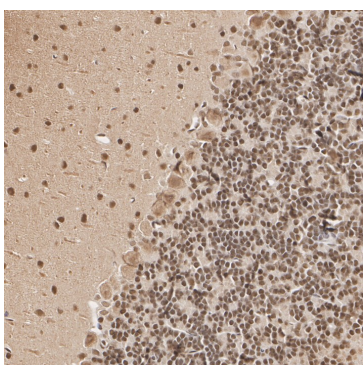
**Fig10:** Immunohistochemical analysis of paraffin-embedded human cerebellum tissue with Rabbit anti-CREB antibody (HA750007) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750007) at 1/5,000 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.



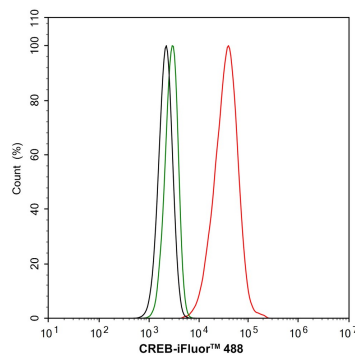
**Fig11:** Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-CREB antibody (HA750007) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750007) at 1/5,000 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.



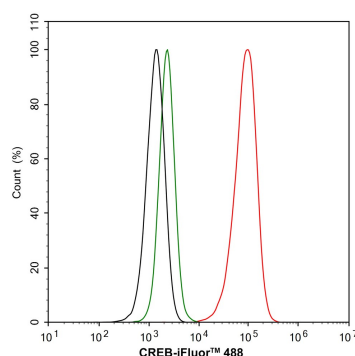
**Fig12:** Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-CREB antibody (HA750007) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750007) at 1/5,000 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.



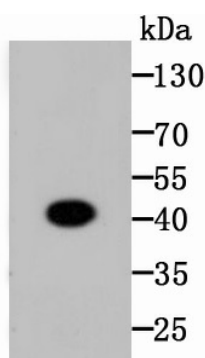
**Fig13:** Flow cytometric analysis of NIH/3T3 cells labeling CREB.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750007, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).



**Fig14:** Flow cytometric analysis of C6 cells labeling CREB.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750007, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).



**Fig15:** Western blot analysis of CREB on zebrafish tissue lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (HA750007, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

**Note:** All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

### Background References

1. Liu F, et al. The oncoprotein HBXIP enhances angiogenesis and growth of breast cancer through modulating FGF8 and VEGF. *Carcinogenesis* 35:1144-53 (2014).
2. Liu L, et al. Genetic deletion of calcium/calmodulin-dependent protein kinase kinase  $\beta$  (CaMKK  $\beta$ ) or CaMK IV exacerbates stroke outcomes in ovariectomized (OVXed) female mice. *BMC Neurosci* 15:118 (2014).

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