abcam

Product datasheet

Anti-CREBBP antibody [EPR23418-23] - ChIP Grade ab253202

Recombinant RabMAb

16 Images

Overview

Product name	Anti-CREBBP antibody [EPR23418-23] - ChIP Grade
Description	Rabbit monoclonal [EPR23418-23] to CREBBP - ChIP Grade
Host species	Rabbit
Specificity	This antibody does not react with mouse species for ChIP application.
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, ChIP, Flow Cyt (Intra), ChIC/CUT&RUN-seq, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, HeLa, 293T, NIH/3T3 and PC-12 whole cell lysate; His-tagged human CREBBP recombinant protein. IHC-P: Human bladder cancer, Human cervical cancer, Mouse cerebrum and Rat cerebrum tissue. ICC: HeLa and NIH/3T3 cells. Flow Cyt: HeLa and NIH/3T3 cells. IP: NIH/3T3 and HeLa cells. ChIP: HeLa cells. ChIC/CUT&RUN-Seq: HeLa cells.
General notes	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23418-23
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab253202 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
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Predicted molecular weight: 51 kDa.
ICC/IF		1/100.
ChIP		Use a concentration of 5 µg/ml.
Flow Cyt (Intra)		1/500.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
IP		1/30.

Target

Function Acetylates histones, giving a specific tag for transcriptional activation. Also acetylates non-histone proteins, like NCOA3 coactivator. Binds specifically to phosphorylated CREB and enhances its transcriptional activity toward cAMP-responsive genes. Acts as a coactivator of ALX1 in the presence of EP300.

Involvement in disease Note=Chromosomal aberrations involving CREBBP may be a cause of acute myeloid leukemias. Translocation t(8;16)(p11;p13) with MYST3/MOZ; translocation t(11;16)(q23;p13.3) with MLL/HRX; translocation t(10;16)(q22;p13) with MYST4/MORF. MYST3-CREBBP may induce leukemia by inhibiting RUNX1-mediated transcription.
Defects in CREBBP are a cause of Rubinstein-Taybi syndrome type 1 (RSTS1) [MIM:180849]. RSTS1 is an autosomal dominant disorder characterized by craniofacial abnormalities, broad thumbs, broad big toes, mental retardation and a propensity for development of malignancies.

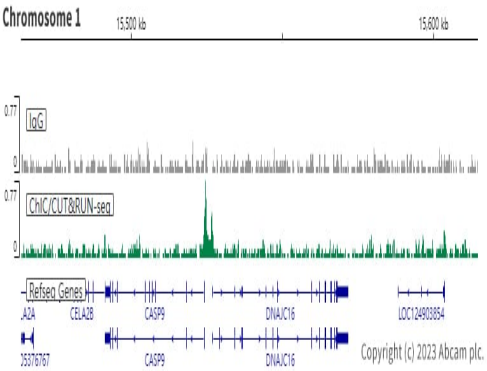
Sequence similarities Contains 1 bromo domain.
Contains 1 KIX domain.
Contains 2 TAZ-type zinc fingers.
Contains 1 ZZ-type zinc finger.

Domain The KIX domain mediates binding to HIV-1 Tat.

Post-translational modifications Methylation of the KIX domain by CARM1 blocks association with CREB. This results in the blockade of CREB signaling, and in activation of apoptotic response.

	Phosphorylated upon DNA damage, probably by ATM or ATR. Sumoylation negatively regulates transcriptional activity via the recruitment of DAAX.
Cellular localization	Cytoplasm. Nucleus. Recruited to nuclear bodies by SS18L1/CREST. In the presence of ALX1 relocalizes from the cytoplasm to the nucleus.

Images

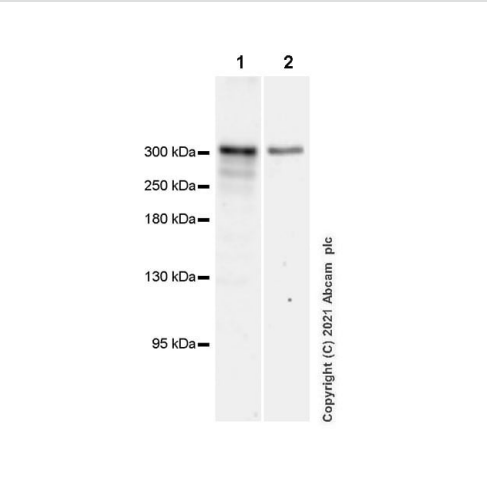


ChIC/CUT&RUN sequencing - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 5µg of ab253202 [EPR23418-23]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

All lanes : Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202) at 1/1000 dilution

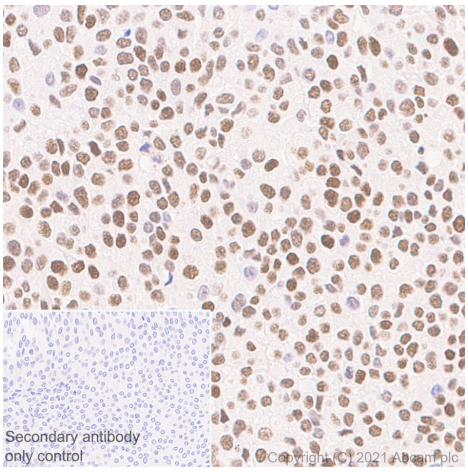
Lane 1 : HAP1 (human chronic myelogenous leukemia near-haploid cell), whole cell lysate at 28 µg
Lane 2 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 14 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 51 kDa
Observed band size: 300 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
Fresh lysates were used in this WB.

Exposure time: Lane 1: 81 seconds; Lane 2: 3 minutes.

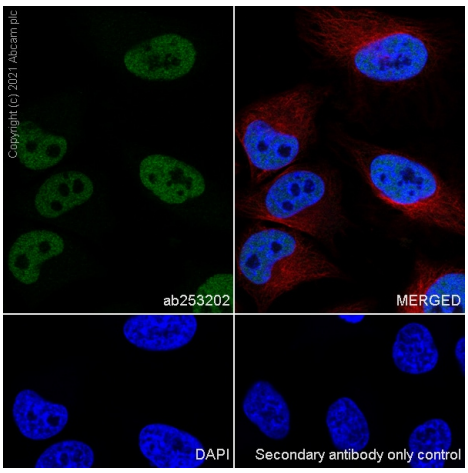


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

Immunohistochemical analysis of paraffin-embedded Human bladder cancer tissue labelling CREBBP with ab253202 at 1/2000 (0.258 µg/mL) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Nuclear staining in human bladder cancer (PMID: 25915404). The section was incubated with ab253202 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 LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

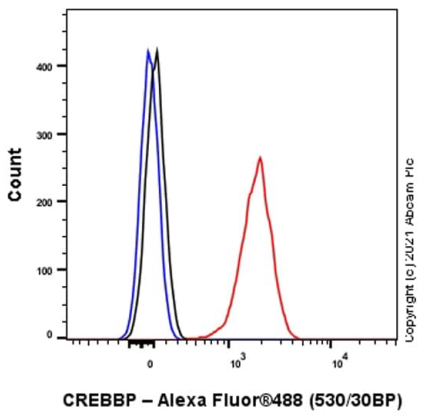
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

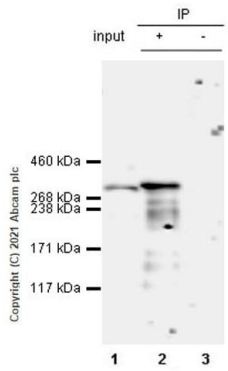
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling CREBBP with ab253202 at 1/500 (1.032 µg/mL) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 µg/mL) dilution (Green). Confocal image showing mianly nuclear staining in HeLa cell line is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 µg/mL) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 µg/mL) dilution.



Flow Cytometry (Intracellular) - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling CREBBP with ab253202 at 1/500 dilution (0.1µg)/ red (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

CREBBP was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast), whole cell lysate 10 µg with ab253202 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab253202 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: NIH/3T3 (mouse embryonic fibroblast), whole cell lysate 10 ug

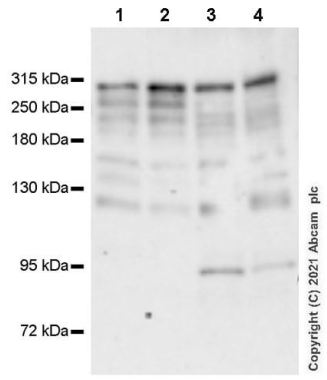
Lane 2: ab253202 IP in NIH/3T3 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab253202 in NIH/3T3 whole cell lysate

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

Exposure time: 110 seconds.

Fresh lysates were used in this IP.



Western blot - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

All lanes : Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : 293T (human embryonic kidney epithelial cell), whole cell lysate
Lane 3 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate
Lane 4 : PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate

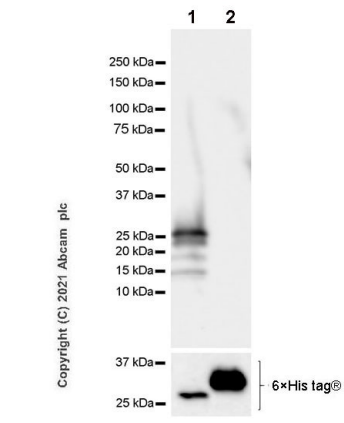
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 51 kDa
Observed band size: 300 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
Fresh lysates were used in this WB.

Exposure time: 81 seconds.



Western blot - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

All lanes : Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202) at 1/1000 dilution

Lane 1 : His-tagged human CREBBP recombinant protein (aa2221-2442)
Lane 2 : His-tagged human EP300 recombinant protein (aa2215-2414)

Lysates/proteins at 0.01 µg per lane.

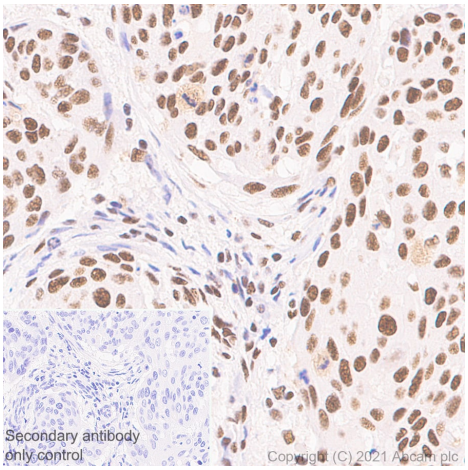
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 51 kDa
Observed band size: 25 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST. This antibody has no cross-reaction with human EP300.

These two recombinant proteins were made in-house and expressed from the E.coli expression system.

Exposure time: 1 second.

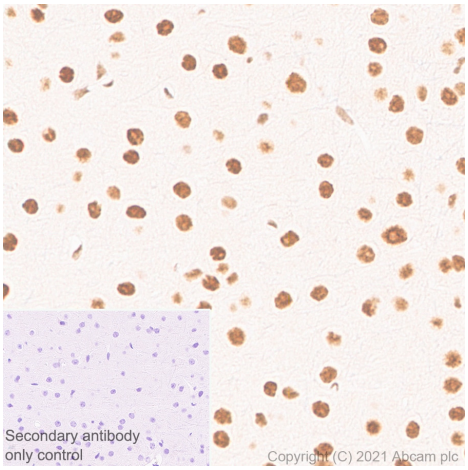


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREBBP antibody [EPR23418-23] (ab253202)

Immunohistochemical analysis of paraffin-embedded Human cervical cancer tissue labelling CREBBP with ab253202 at 1/2000 (0.258 µg/mL) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Nuclear staining in human cervical cancer. The section was incubated with ab253202 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 LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

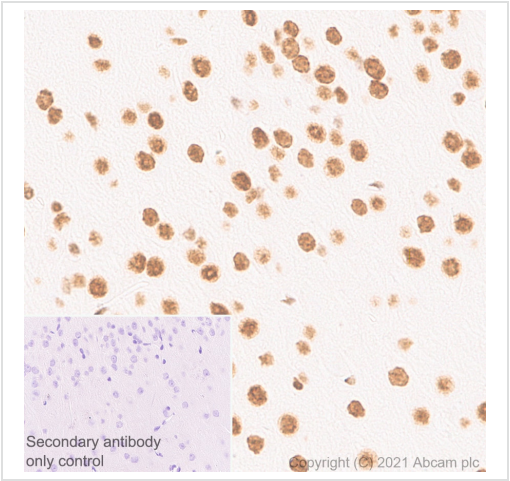


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labelling CREBBP with ab253202 at 1/2000 (0.258 µg/mL) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Mainly nuclear staining in mouse cerebrum. The section was incubated with ab253202 for 10 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 LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

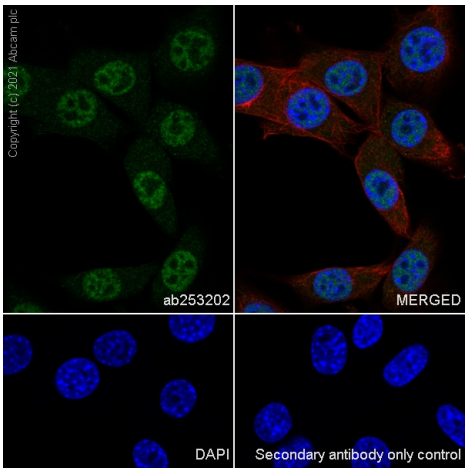


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labelling CREBBP with ab253202 at 1/2000 (0.258 µg/mL) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Mainly nuclear staining in rat cerebrum (PMID: 12445700). The section was incubated with ab253202 for 10 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 LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

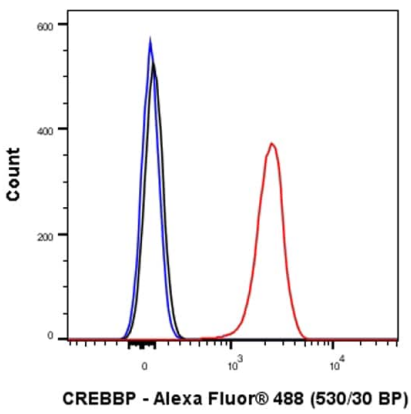
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

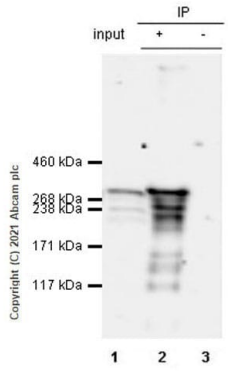
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 cells labelling CREBBP with ab253202 at 1/100 (5.16 µg/mL) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 µg/mL) dilution (Green). Confocal image showing mianly nuclear staining in NIH/3T3 cell line is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 µg/mL) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 µg/mL) dilution.



Flow Cytometry (Intracellular) - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labelling CREBBP with ab253202 at 1/500 dilution (0.1µg)/ red (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

CREBBP was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate 10 µg with ab253202 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab253202 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg

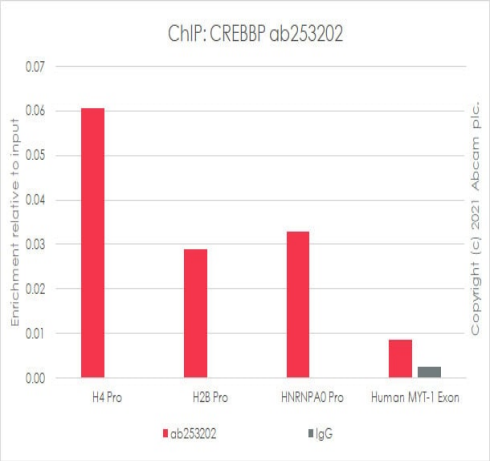
Lane 2: ab253202 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab253202 in HeLa whole cell lysate

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

Exposure time: 48 seconds.

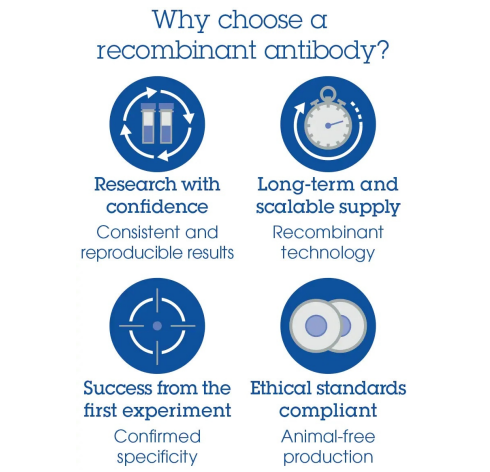
Fresh lysates were used in this IP.



ChIP - Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

Chromatin was prepared from HeLa cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab253202 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).
Primers are from paper PMID:2178921

* http://www.abcam.com/resources?keywords=X%20ChIP%20protocol



Anti-CREBBP antibody [EPR23418-23] - ChIP Grade (ab253202)

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