abcam

Product datasheet

Anti-ATF6 antibody [EPR22690-84] - ChIP Grade ab227830

KO VALIDATED Recombinant RabMAb

3 Abreviews 9 References 8 Images

Overview

Product name	Anti-ATF6 antibody [EPR22690-84] - ChIP Grade
Description	Rabbit monoclonal [EPR22690-84] to ATF6 - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ChIP, IP, ChIC/CUT&RUN-seq **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1 and HeLa whole cell lysates. IHC-P: Human kidney tissue. IP: HeLa whole cell lysate. ChIP: Chromatin prepared from RAW 264.7 (treated with tunicamycin) and HeLa (treated with thapsigargin) 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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR22690-84
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227830 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	★★★★☆ (1)	1/1000. Predicted molecular weight: 74 kDa.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ChIP		Use 5 µg for 25 µg of chromatin.
IP		1/30.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg

Application notes Is unsuitable for Flow Cyt or ICC/IF.

Target

Function Transcription factor that acts during endoplasmic reticulum stress by activating unfolded protein response target genes. Binds DNA on the 5'-CCAC[GA]-3'half of the ER stress response element (ERSE) (5'-CCAAT-N(9)-CCAC[GA]-3') and of ERSE II (5'-ATTGG-N-CCACG-3'). Binding to ERSE requires binding of NF-Y to ERSE. Could also be involved in activation of transcription by the serum response factor.

Tissue specificity Ubiquitous.

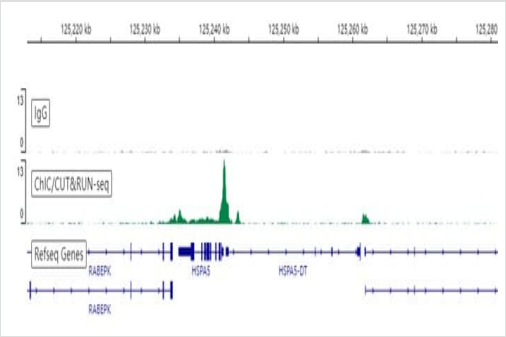
Sequence similarities Belongs to the bZIP family. ATF subfamily.
Contains 1 bZIP domain.

Domain The basic domain functions as a nuclear localization signal.
The basic leucine-zipper domain is sufficient for association with the NF-Y trimer and binding to ERSE.

Post-translational modifications During unfolded protein response an approximative 50 kDa fragment containing the cytoplasmic transcription factor domain is released by proteolysis. The cleavage seems to be performed sequentially by site-1 and site-2 proteases.
N-glycosylated. The glycosylation status may serve as a sensor for ER homeostasis, resulting in ATF6 activation to trigger the unfolded protein response (UPR).
Phosphorylated in vitro by MAPK14/P38MAPK.

Cellular localization Endoplasmic reticulum membrane and Nucleus. Under ER stress the cleaved N-terminal cytoplasmic domain translocates into the nucleus.

Images

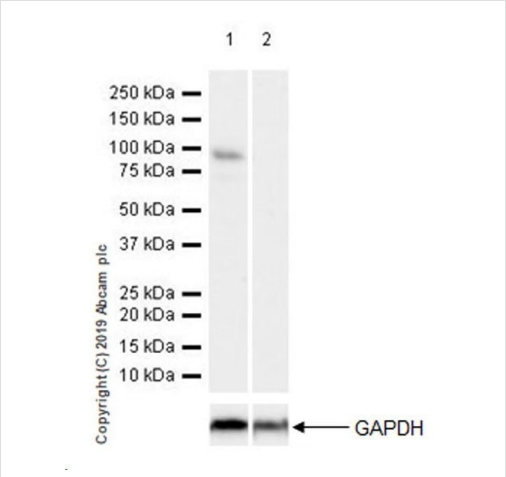


ChIC/CUT&RUN sequencing - Anti-ATF6 antibody [EPR22690-84] - ChIP Grade (ab227830)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 HeLa cells treated with Thapsigargin (0.5 µM for 24h) and 5µg of ab227830 [EPR22690-84]. 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-ATF6 antibody [EPR22690-84] (ab227830)

All lanes : Anti-ATF6 antibody [EPR22690-84] - ChIP Grade (ab227830) at 1/1000 dilution

Lane 1 : Wild type HAP1 whole cell lysate
Lane 2 : ATF6 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

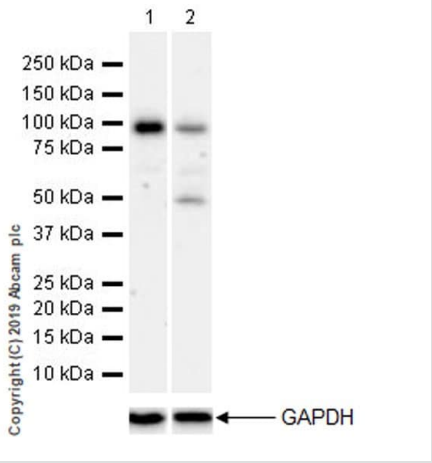
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 74 kDa
Observed band size: 90 kDa

ab227830 was shown to specifically react with ATF6 in wild-type HAP1 cells as signal was lost in ATF6 knockout cells. Wild-type and ATF6 knockout samples were subjected to SDS-PAGE. ab227830 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/20,000 dilution for 1 hour at room temperature before imaging.

Exposure time 62 seconds.

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



Western blot - Anti-ATF6 antibody [EPR22690-84] (ab227830)

All lanes : Anti-ATF6 antibody [EPR22690-84] - ChIP Grade (ab227830) at 1/1000 dilution

Lane 1 : Untreated HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa treated with 1 µ? thapsigargin for 1 hour, whole cell lysate

Lysates/proteins at 10 µg per lane.

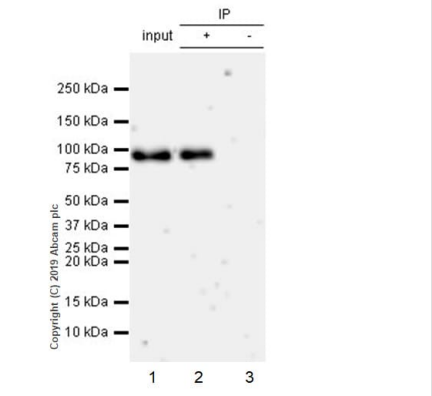
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 74 kDa
Observed band size: 50,90 kDa

ATF6 is cleaved upon ER stress and the molecular weight observed is consistent with what has been described in the literature (PMID: 25149687; 11163209).

Exposure time 3 minutes.

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



Immunoprecipitation - Anti-ATF6 antibody [EPR22690-84] (ab227830)

ATF6 was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate with ab227830 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab227830 1/1000 dilution (0.5 µg/ml). VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

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

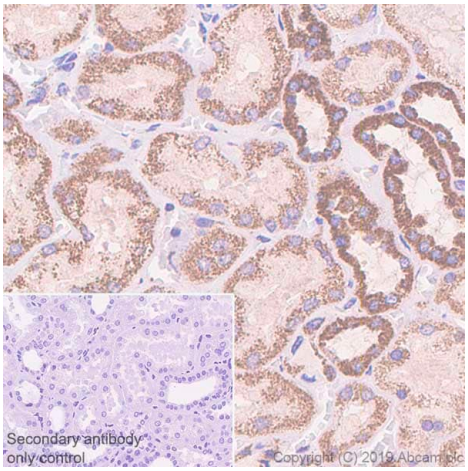
Lane 2: **ab254324** IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab227830 in HeLa whole cell lysate.

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

Exposure time: 3 min.

Lysate were made freshly and used in IP test immediately to minimize protein degradation. Incubation time was 2h.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATF6 antibody [EPR22690-84] (ab227830)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling ATF6 with ab227830 at 1/1000 dilution (0.566 µg/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human kidney (PMID: 25725420) is observed. The section was incubated with ab227830 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins.

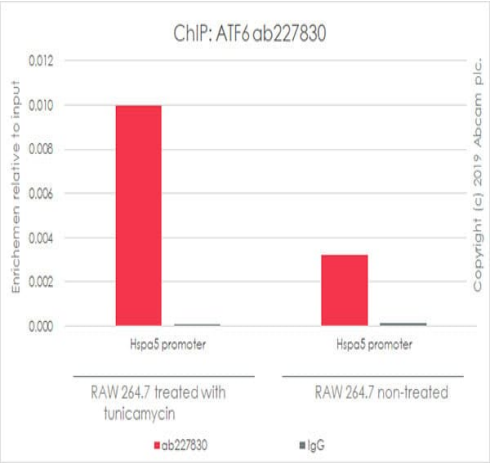
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

ChIP - Anti-ATF6 antibody [EPR22690-84] (ab227830)

Chromatin was prepared from HeLa cells treated with thapsigargin (1 uM, 1 h) according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30 mins and then formaldehyde for 10 min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab227830 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are from paper PMID: 17535801.

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



ChIP - Anti-ATF6 antibody [EPR22690-84] (ab227830)

Chromatin was prepared from RAW 264.7 cells treated with tunicamycin (5 ug/ml, 4 h) according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30 mins and then formaldehyde for 10 min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab227830 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are from paper PMCID: PMC5179193.

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

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