abcam

Product datasheet

Anti-ATF3 antibody [EPR19488] - ChIP Grade ab207434

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 30 References 15 Images

Overview

Product name	Anti-ATF3 antibody [EPR19488] - ChIP Grade
Description	Rabbit monoclonal [EPR19488] to ATF3 - ChIP Grade
Host species	Rabbit
Specificity	Stimulation may be required to allow detection of the target protein due to low levels of endogenous expression in some samples. Please see images below for recommended treatment conditions and positive controls.
Tested applications	**Suitable for:** ChIC/CUT&RUN-seq, ChIP, WB, IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HCT116, HEK-293, HeLa, A431, HepG2, LnCap, Jurkat, THP-1, RAW 264.7 cell lysates. ICC/IF: THP-1, RAW 264.7, HAP1 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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR19488
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab207434 in the following tested applications.

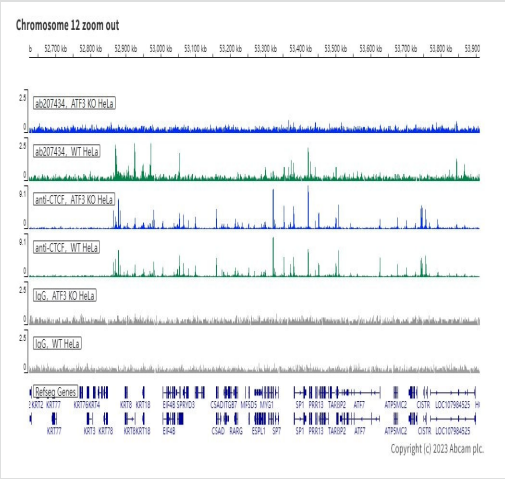
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
WB		1/1000. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).
IP		1/50.
ICC/IF		1/100.

Target

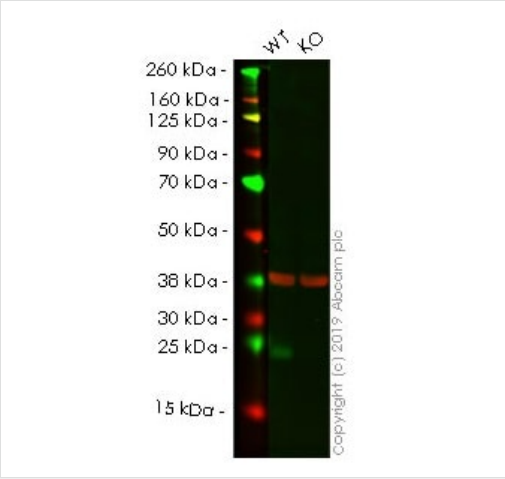
Function	This protein binds the cAMP response element (CRE) (consensus: 5'-GTGACGT[AC][AG]-3'), a sequence present in many viral and cellular promoters. Represses transcription from promoters with ATF sites. It may repress transcription by stabilizing the binding of inhibitory cofactors at the promoter. Isoform 2 activates transcription presumably by sequestering inhibitory cofactors away from the promoters.
Sequence similarities	Belongs to the bZIP family. ATF subfamily. Contains 1 bZIP domain.
Cellular localization	Nucleus.

Images



ChIC/CUT&RUN sequencing - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/µL. 2.5X10^5 of Human ATF3 knockout HeLa cell line (**ab264908**) or Human wild-type HeLa cell line (**ab255448**) were used along with 5µg of Anti-ATF3 antibody (ab207434). Assay Quality Control was conducted using 5µg Anti-CTCF (**ab188408**) on the same cell lines. 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-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

All lanes : Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATF3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

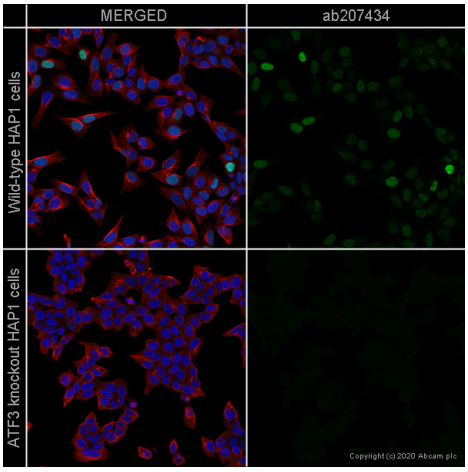
Performed under reducing conditions.

Predicted band size: 21 kDa
Observed band size: 21 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab207434 observed at 21 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab207434 was shown to react with ATF3 in wild-type HeLa cells in

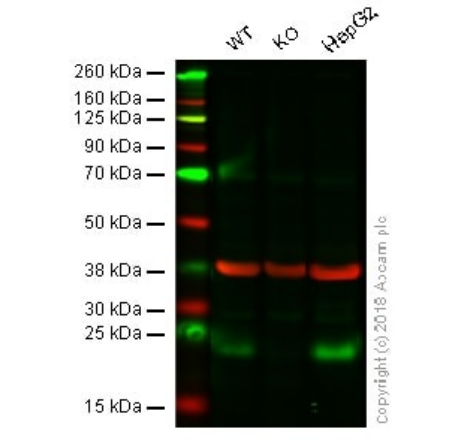
western blot. Loss of signal was observed when knockout cell line **ab264908** (knockout cell lysate **ab257073**) was used. Wild-type HeLa and ATF3 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab207434 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

ab207434 staining ATF3 in wild-type Hap1 cells (top panel) and ATF3 knockout Hap1 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab207434 at 1/100 dilution and **ab7291** (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Western blot - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

All lanes : Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : ATF3 knockout HAP1 whole cell lysate
Lane 3 : HepG2 whole cell lysate

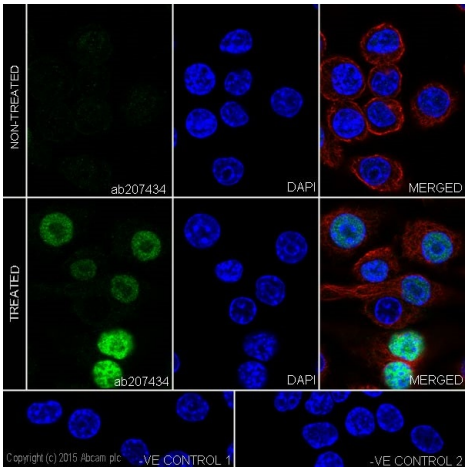
Lysates/proteins at 20 µg per lane.

Predicted band size: 21 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab207434 observed at 21 kDa. Red - loading control, **ab9484**, observed at 37

kDa.

ab207434 was shown to specifically react with ATF3 in wild-type HAP1 cells as signal was lost in ATF3 knockout cells. Wild-type and ATF3 knockout samples were subjected to SDS-PAGE. ab207434 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

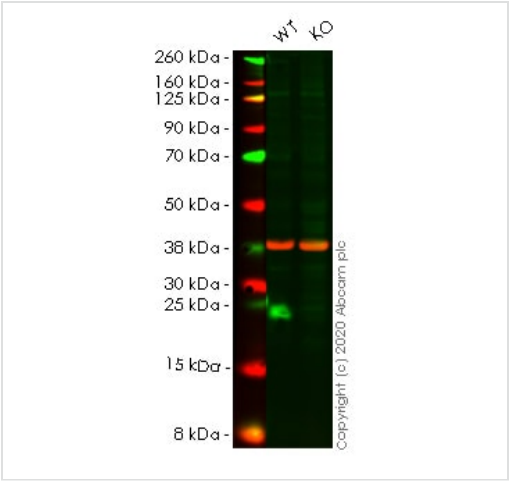


Immunocytochemistry/ Immunofluorescence - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cells labeling ATF3 with ab207434 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing increased nuclear staining on RAW 264.7 cell line, after treatment with LPS (1µg/ml) for 2 hours.

The nuclear counter stain is DAPI (blue). Tubulin is detected with with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab207434 at 1/100 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution.
-ve control 2: with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Western blot - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

All lanes : Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : ATF3 knockout HCT116 cell lysate

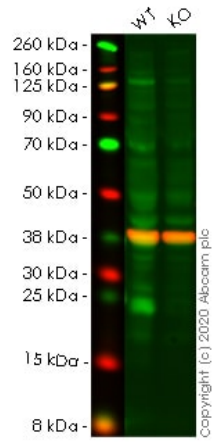
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21 kDa
Observed band size: 21 kDa

Lanes 1-2: Merged signal (red and green). Green - ab207434 observed at 21 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab207434 Anti-ATF3 antibody [EPR19488] - ChIP Grade was shown to specifically react with ATF3 in wild-type HCT116 cells. Loss of signal was observed when knockout cell line **ab266872** (knockout cell lysate **ab257074**) was used. Wild-type and ATF3 knockout samples were subjected to SDS-PAGE. ab207434 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

All lanes : Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : ATF3 knockout A549 cell lysate

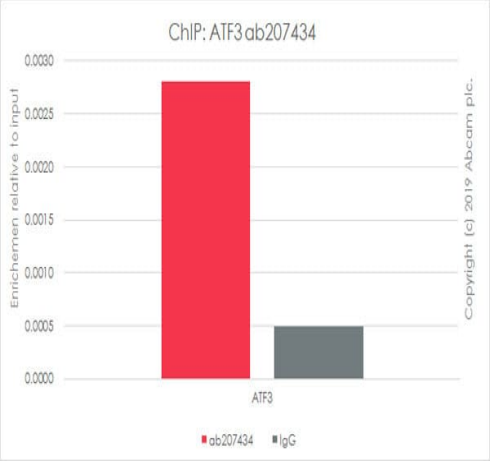
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21 kDa
Observed band size: 21 kDa

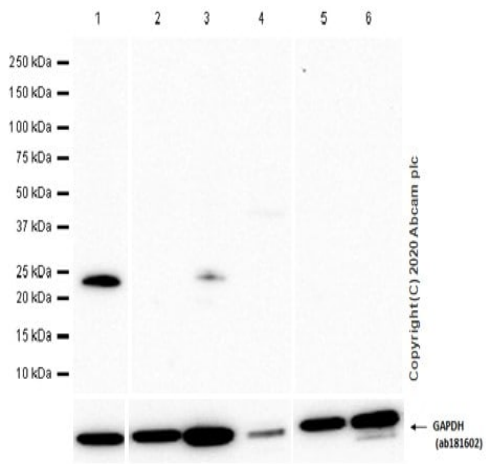
Lanes 1-2: Merged signal (red and green). Green - ab207434 observed at 21 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab207434 Anti-ATF3 antibody [EPR19488] - ChIP Grade was shown to specifically react with ATF3 in wild-type A549 cells. Loss of signal was observed when knockout cell line **ab266955** (knockout cell lysate **ab257075**) was used. Wild-type and ATF3 knockout samples were subjected to SDS-PAGE. ab207434 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



ChIP - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

Chromatin was prepared from Hela cells according to the Abcam Dual X-ChIP protocol*. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab207434 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).
Primers and probes are located in the first kb of the transcribed region.
*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol



Western blot - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

All lanes : Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/500 dilution

Lane 1 : 293T (Human embryonic kidney epithelial cell) whole cell lysate
Lane 2 : Human liver tissue lysate
Lane 3 : Raw264.7 (Mouse abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 4 : Mouse liver tissue lysate
Lane 5 : MEF (Mouse embryonic fibroblast (immortalized)) whole cell lysate
Lane 6 : Mouse heart tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 21 kDa
Observed band size: 21 kDa

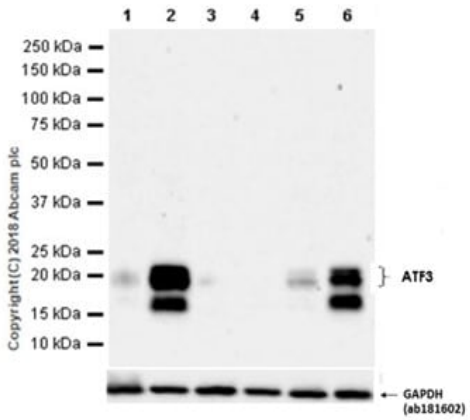
Exposure time: 180 seconds

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

ATF3 has a low expression level in some cell lines and tissues, but is increased under treatment (PMID: 8622660, PMID: 22053207,

PMID: 20018623, PMID: 29940414).

Rabbit monoclonal [EPR16891] to GAPDH (**ab181602**) used as loading control.



Western blot - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

All lanes : Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate
Lane 4 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate
Lane 5 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate
Lane 6 : LnCap (Human prostate carcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

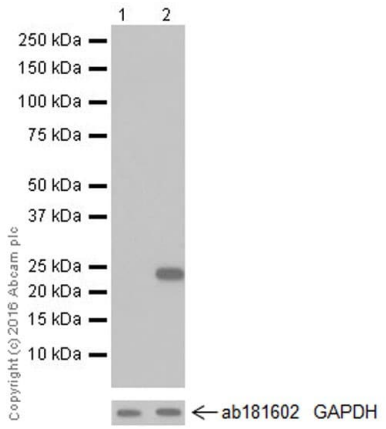
Predicted band size: 21 kDa
Observed band size: 21,23 kDa

Exposure time: 180 seconds

Blocking/Diluting buffer and concentration 5% NFDM /TBST

ATF3 migrates as a 21 and 23 kDa doublet band due to an alternative ATG usage (PMID: 12225289, PMID: 8649793)

The mRNA and protein expression of ATF3 is low or undetectable in most cells, but its expression is rapidly induced by a large variety of cellular stresses including DNA damage, wounds, and cellular injury (PMID: 19136462, 20651982, 20592017).



Western blot - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

All lanes : Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/1000 dilution

Lane 1 : Untreated THP-1 (Human monocytic leukemia cell line) whole cell lysate
Lane 2 : THP-1 (Human monocytic leukemia cell line) treated with 80nM TPA overnight, then treated with 1 µg/ml LPS for 8 hours whole cell lysate

Lysates/proteins at 10 µg per lane.

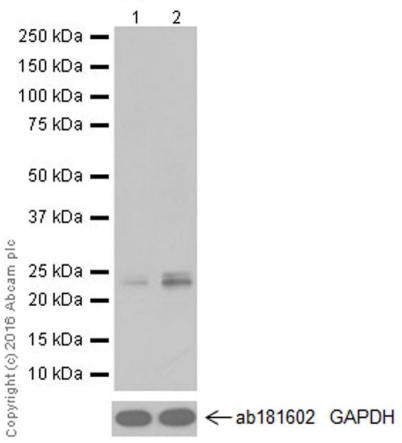
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 21 kDa
Observed band size: 21 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID: 24973221).



Western blot - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

All lanes : Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/1000 dilution

Lane 1 : Untreated RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 2 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) treated with 1 µg/ml LPS for 2 hours whole cell lysate

Lysates/proteins at 10 µg per lane.

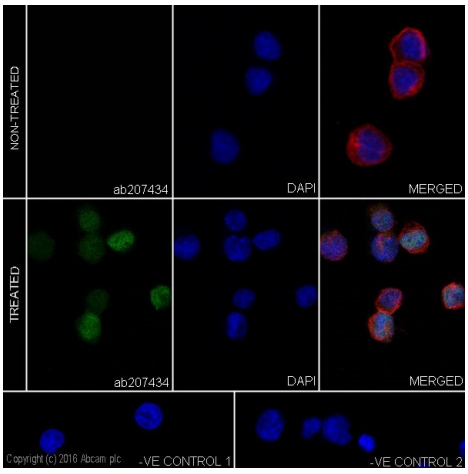
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 21 kDa
Observed band size: 21 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID: 24973221, PMID: 19136462).

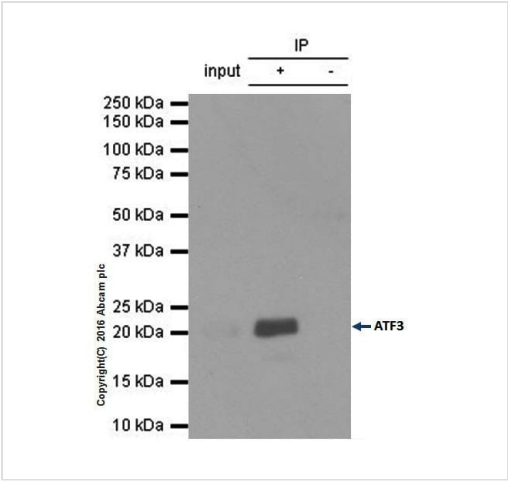


Immunocytochemistry/ Immunofluorescence - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized THP-1 (Human monocytic leukemia cell line) cells labeling ATF3 with ab207434 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing increased nuclear staining on THP-1 cell line, after treatment with TPA (80nM) for overnight, followed by LPS (1µg/ml) for 8 hours.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab207434 at 1/100 dilution followed by followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

ab207434 at 1/50 immunoprecipitating ATF3 in HeLa (human cervix adenocarcinoma) cells.

Lane 1 (input): HeLa whole cell lysate 10µg
Lane 2 (+): ab207434 + HeLa whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab207434 in HeLa (human cervix adenocarcinoma) whole cell lysate

For western blotting, ab207434 (1:500) as primary antibody and VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

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

Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434)

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors