abcam

Product datasheet

Anti-ATF6 antibody ab83504

2 References 5 Images

Overview

Product name	Anti-ATF6 antibody
Description	Rabbit polyclonal to ATF6
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Cow
Immunogen	Synthetic peptide corresponding to Human ATF6 aa 650 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as ab92653)
Positive control	WB: HeLa, Hap1 and MDA-MB-361 cell lysates. ICC/IF: HeLa cells.
General notes	Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee. In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for. We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee. Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab83504** in the following tested applications.

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

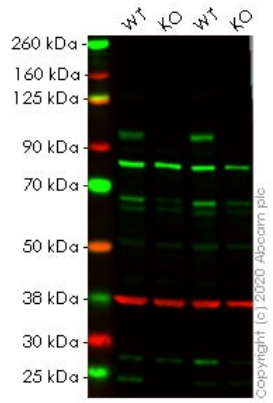
Application	Abreviews	Notes
ICC/IF		Use a concentration of 1 µg/ml.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 75,90,100 kDa (predicted molecular weight: 75 kDa).

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



Western blot - Anti-ATF6 antibody (ab83504)

All lanes : Anti-ATF6 antibody (ab83504)

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATF6 knockout HeLa cell lysate
Lane 3 : Wild-type HAP1 cell lysate
Lane 4 : ATF6 knockout HAP1 cell lysate

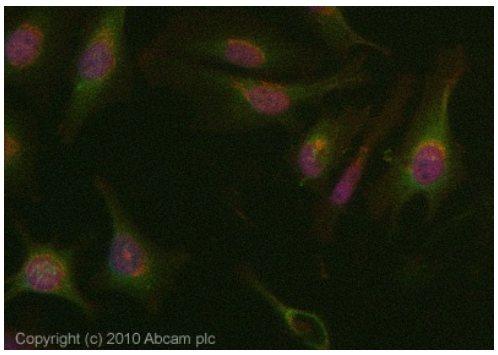
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 75 kDa

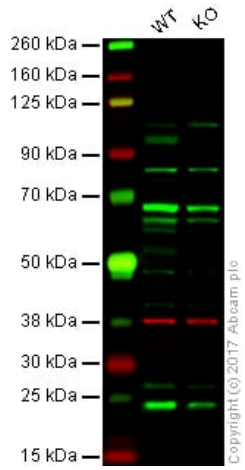
Lanes 1-4: Merged signal (red and green). Green - ab83504 observed at 95 kDa. Red - loading control ab8245 observed at 37 kDa.

ab83504 Anti-ATF6 antibody was shown to specifically react with ATF6 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261800 (knockout cell lysate ab256841) was used. Wild-type and ATF6 knockout samples were subjected to SDS-PAGE. ab83504 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 µg/ml 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.



Immunocytochemistry/ Immunofluorescence - Anti-ATF6 antibody (ab83504)

ICC/IF image of ab83504 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal Goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab83504, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 Goat anti-Rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-ATF6 antibody (ab83504)

All lanes : Anti-ATF6 antibody (ab83504) at 1 µg/ml

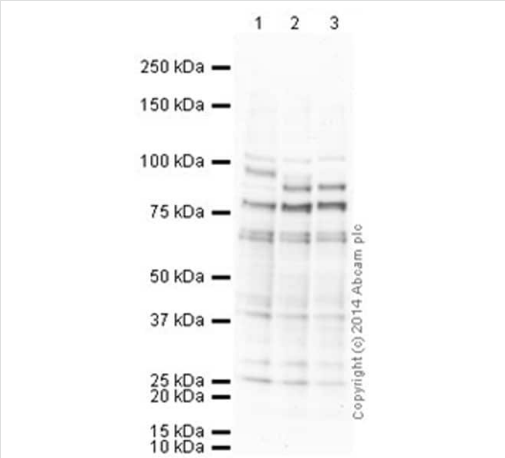
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : ATF6 knockout HAP1 whole cell lysate

Lysates/proteins at 50 µg per lane.

Predicted band size: 75 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab83504 observed at 95 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab83504 was shown to recognize ATF6 in wild-type HAP1 cells along with additional cross reactive bands . No bands were observed when ATF6 knockout samples were used. Wild-type and ATF6 knockout samples were subjected to SDS-PAGE. Ab83504 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at a concentration of 1 ug/ml and 1/10,000 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 1/10,000 dilution for 1hr at room temperature before imaging.



Western blot - Anti-ATF6 antibody (ab83504)

All lanes : Anti-ATF6 antibody (ab83504) at 1 µg/ml

Lane 1 : HeLa Whole Cell Lysate (untreated)
Lane 2 : HeLa Whole Cell Lysate (treated with 2.37 uM Tunicamycin)
Lane 3 : HeLa Whole Cell Lysate (treated with 23.7 uM Tunicamycin)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 75 kDa
Observed band size: 75,90 kDa
why is the actual band size different from the predicted?
Additional bands at: 100 kDa (possible glycosylated form)

Exposure time: 8 minutes

ATF6 has a predicted molecular weight of 75 kDa; however it has a number of potential glycosylation sites which may affect the migration of the protein (SwissProt data). We believe the bands observed at 75 and 100 kDa correspond to the full length nonglycosylated and glycosylated forms of ATF6. Following Tunicamycin treatment to inhibit N-linked glycosylation we see the appearance of a partially deglycosylated form at 90 kDa.

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab83504 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406

Western blot - Anti-ATF6 antibody (ab83504)

Anti-ATF6 antibody (ab83504) at 1 µg/ml + MDA-MB-361 (Human breast adenocarcinoma cell line) Whole Cell Lysate at 10 µg

Secondary

Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 75 kDa
Observed band size: 75,78 kDa why is the actual band size different from the predicted?
Additional bands at: 60 kDa, 90 kDa. We are unsure as to the identity of these extra bands.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

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