# abcam

### Product datasheet

## Anti-ATF1 antibody [EPR17028] ab181569

Recombinant RabMAb

#### 10 Images 2 References

#### Overview

**Product name** Anti-ATF1 antibody [EPR17028]

**Description** Rabbit monoclonal [EPR17028] to ATF1

**Host species** Rabbit

**Tested applications** Suitable for: IP, WB, IHC-P, ICC/IF, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse heart and kidney lysates; Rat heart lysate; HeLa, C6, RAW 264.7 and NIH/3T3 whole

cell lysates. IHC-P: Mouse liver tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa

cells. IP: Mouse kidney lysate.

**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

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long Storage instructions

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR17028

Isotype lgG

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab181569 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
IP		1/50.
WB		1/1000. Detects a band of approximately 35 kDa (predicted molecular weight: 29 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. This antibody is not suitable for human and rat species in IHC application.
ICC/IF		1/100.
Flow Cyt (Intra)		1/150.

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**Function** 

This protein binds the cAMP response element (CRE) (consensus: 5'-GTGACGT[AC][AG]-3'), a sequence present in many viral and cellular promoters. Binds to the Tax-responsive element (TRE) of HTLV-I. Mediates PKA-induced stimulation of CRE-reporter genes.

Involvement in disease

Defects in ATF1 may be a cause of angiomatoid fibrous histiocytoma (AFH) [MIM:612160]. A distinct variant of malignant fibrous histiocytoma that typically occurs in children and adolescents and is manifest by nodular subcutaneous growth. Characteristic microscopic features include lobulated sheets of histiocyte-like cells intimately associated with areas of hemorrhage and cystic pseudovascular spaces, as well as a striking cuffing of inflammatory cells, mimicking a lymph node metastasis. Note=Chromosomal aberrations involving ATF1 are found in patients with angiomatoid fibrous histiocytoma. Translocation t(12;16)(q13;p11.2) with FUS generates a chimeric ATF1/FUS protein. Translocation t(12;22)(q13;q12) with EWSR1 generates a chimeric ATF1/EWSR1 protein.

Sequence similarities

Belongs to the bZIP family. ATF subfamily.

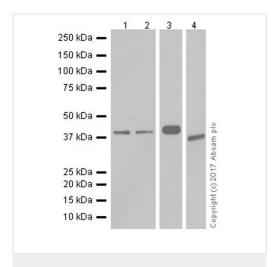
Contains 1 bZIP domain.

Contains 1 KID (kinase-inducible) domain.

**Cellular localization** 

Nucleus.

#### **Images**



Western blot - Anti-ATF1 antibody [EPR17028] (ab181569)

**All lanes :** Anti-ATF1 antibody [EPR17028] (ab181569) at 1/1000 dilution

Lane 1 : Mouse heart lysate

Lane 2: Mouse kidney lysate

Lane 3: Rat heart lysate

Lane 4: HeLa (human epithelial cell line from cervix

adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

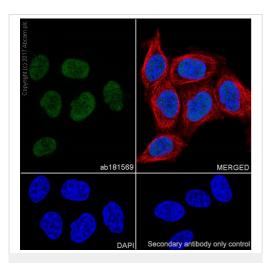
Developed using the ECL technique.

**Predicted band size:** 29 kDa **Observed band size:** 35 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID:10574952).

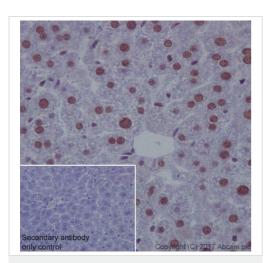


Immunocytochemistry/ Immunofluorescence - Anti-ATF1 antibody [EPR17028] (ab181569)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling ATF1 with ab181569 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (**ab150077**) secondary antibody at 1/1000 dilution.

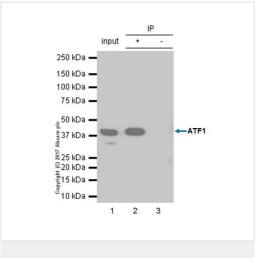


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ATF1 antibody
[EPR17028] (ab181569)

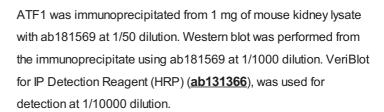
Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling ATF1 with ab181569 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining on mouse liver (PMID: 11865068; PMID: 28032861). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Immunoprecipitation - Anti-ATF1 antibody [EPR17028] (ab181569)



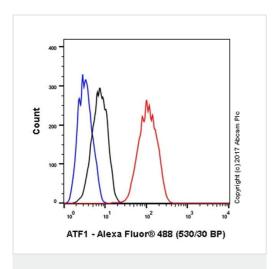
Lane 1: Mouse kidney lysate 10 µg (Input).

Lane 2: ab181569 IP in mouse kidney lysate (+).

Lane 3: Rabbit monoclonal  $\lg G$  ( $\underline{ab172730}$ ) instead of ab181569 in mouse kidney lysate (-).

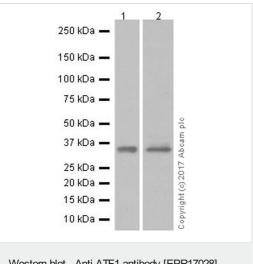
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.



Flow Cytometry (Intracellular) - Anti-ATF1 antibody [EPR17028] (ab181569)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling ATF1 with ab181569 at 1/150 dilution (red) compared with a Rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-ATF1 antibody [EPR17028] (ab181569)

All lanes: Anti-ATF1 antibody [EPR17028] (ab181569) at 1/1000 dilution

Lane 1: C6 (rat glial tumor cell line) whole cell lysate

Lane 2: RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) 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

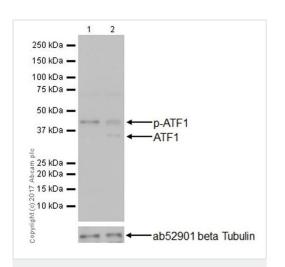
Developed using the ECL technique.

Predicted band size: 29 kDa Observed band size: 35 kDa



Exposure time: Lane 1: 3 minutes; Lane 2: 30 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-ATF1 antibody [EPR17028] (ab181569)

All lanes: Anti-ATF1 antibody [EPR17028] (ab181569) at 1/1000 dilution

Lane 1: Mouse kidney lysate

Lane 2: Mouse kidney lysate treated with alkaline phosphatase for 1 hour at 37°C

Lysates/proteins at 2.5 µg per lane.

#### Secondary

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

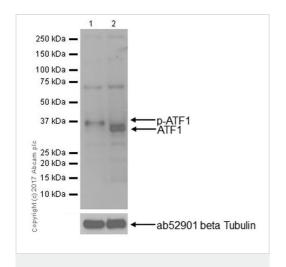
Developed using the ECL technique.

Predicted band size: 29 kDa Observed band size: 35 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

ATF1 has been shown to be phosphorylated in cultured cells and hyperphosphorylation in tissues (PMID:20730097). Treatment of cell lysates with alkaline phosphatase lead to complete dephosphorylation, while we were only able to partial dephosphorylate ATF1 in tissue lysates.



Western blot - Anti-ATF1 antibody [EPR17028] (ab181569)

**All lanes :** Anti-ATF1 antibody [EPR17028] (ab181569) at 1/1000 dilution

**Lane 1**: NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

**Lane 2**: NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate treated with alkaline phosphatase for 1 hour at 37°C

Lysates/proteins at 25 µg per lane.

#### Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

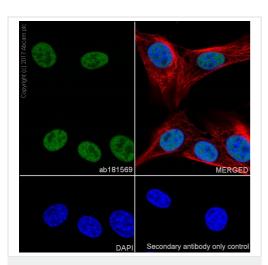
Developed using the ECL technique.

**Predicted band size:** 29 kDa **Observed band size:** 35 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

ATF1 has been shown to be phosphorylated in cultured cells and hyperphosphorylation in tissues (PMID:20730097). Treatment of cell lysates with alkaline phosphatase lead to complete dephosphorylation, while we were only able to partial dephosphorylate ATF1 in tissue lysates.

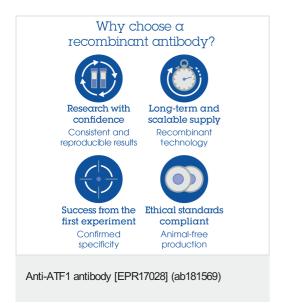


Immunocytochemistry/ Immunofluorescence - Anti-ATF1 antibody [EPR17028] (ab181569)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cells labeling ATF1 with ab181569 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on NIH/3T3 cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.



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