abcam

Product datasheet

Anti-ATF3 antibody [EPR22610-19] - ChIP Grade ab254268

KO 評価済 リコンビナント RabMAb

1 Abreviews 13 References 画像数 14

製品の概要

製品名	Anti-ATF3 antibody [EPR22610-19] - ChIP Grade
製品の詳細	Rabbit monoclonal [EPR22610-19] to ATF3 - ChIP Grade
由来種	Rabbit
特異性	IHC is not recommended for mouse. 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.
アプリケーション	**適用あり:** ChIC/CUT&RUN-seq, Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP, ChIP
種交差性	**交差種:** Mouse, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: THP-1 treated with 80nM TPA overnight, then treated with 1µg/ml lipopolysaccharide (LPS) for 8 hours, whole cell lysate; RAW 264.7 (+/- treated with 1µg/ml lipopolysaccharide (LPS) for 2 hours) whole cell lysate; HepG2, HAP1, A549 and HCT116 whole cell lysates. IHC-P: Human placenta and Hodgkin's lymphoma tissue. ICC/IF: THP-1 cells treated with PMA and LPS. Flow cyt: THP-1 cells treated with PMA and LPS. IP: RAW 264.7 (LPS -treated) whole cell lysate ChIP: Chromatin prepared from HeLa cells.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	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.

バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR22610-19
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab254268の使用に適用されます

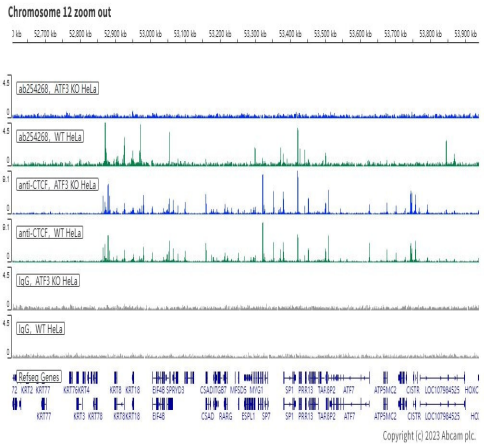
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
Flow Cyt (Intra)		1/600.
WB		1/1000. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. IHC is not recommended for mouse.
ICC/IF		1/100.
IP		1/30.
ChIP		Use 5 µg for 25 µg of chromatin.

ターゲット情報

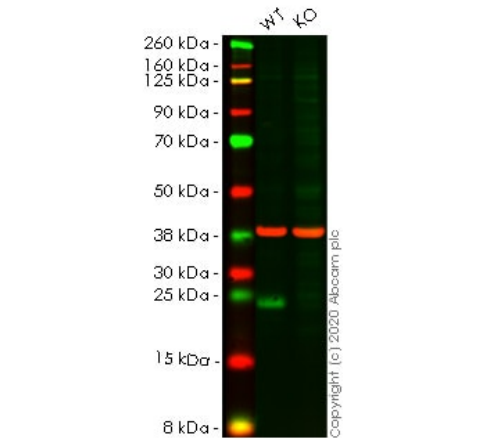
機能	This protein binds the cAMP response element (CRE) (consensus: 5'-GTGACGT[AC][AG]-3'), a sequence present in many viral and cellular promoters. Represses transcription from promoters with ATF sites. It may repress transcription by stabilizing the binding of inhibitory cofactors at the promoter. Isoform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

画像



ChIC/CUT&RUN sequencing - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

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 (ab254268). 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 [EPR22610-19] - ChIP Grade (ab254268)

All lanes : Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268) 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.

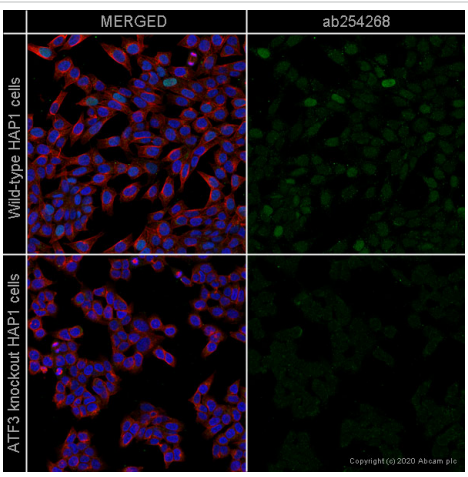
Performed under reducing conditions.

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

Lanes 1-2: Merged signal (red and green). Green - ab254268 observed at 21 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab254268 Recombinant Anti-ATF3 antibody [EPR22610-19] 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. ab254268 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

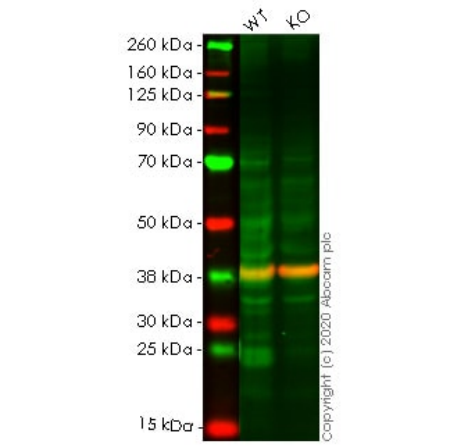
antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

ab254268 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 ab254268 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 [EPR22610-19] - ChIP Grade (ab254268)

All lanes : Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268) 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.

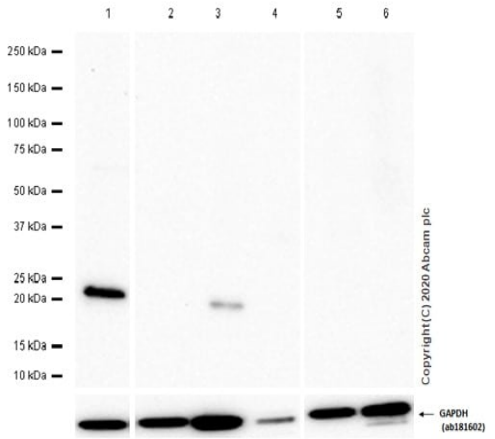
Performed under reducing conditions.

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

Lanes 1-2: Merged signal (red and green). Green - ab254268 observed at 21 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab254268 Recombinant Anti-ATF3 antibody [EPR22610-19] 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. ab254268 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 [EPR22610-19] - ChIP Grade (ab254268)

All lanes : Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268) at 1/1000 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

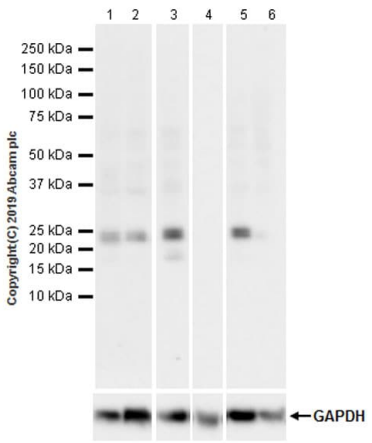
Predicted band size: 21 kDa
Observed band size: 21 kDa

Exposure time: 180 seconds

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

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

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).



Western blot - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

All lanes : Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268) at 1/1000 dilution

Lane 1 : HepG2 (human hepatocellular carcinoma epithelial cell), whole cell lysate
Lane 2 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 3 : HEK-293T (human embryonic kidney epithelial cell), whole cell lysate
Lane 4 : Daudi (human Burkitts lymphoma lymphoblast), whole cell lysate
Lane 5 : Wild-type HAP1 whole cell lysate
Lane 6 : ATF3 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

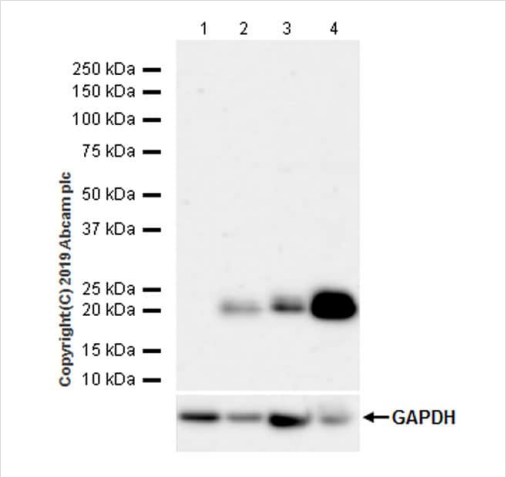
Predicted band size: 21 kDa
Observed band size: 21 kDa

Exposure time: 26 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

Negative control: Daudi (PMID:19136462).

The molecular weight observed is consistent with what has been described in the literature (PMID:18692824).



Western blot - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

All lanes : Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268) at 1/1000 dilution

Lane 1 : Untreated THP-1 (human monocytic leukemia monocyte), whole cell lysate
Lane 2 : THP-1 treated with 80nM TPA overnight, then treated with 1ug/ml lipopolysaccharide (LPS) for 8 hours, whole cell lysate
Lane 3 : Untreated RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate
Lane 4 : RAW264.7 treated with 1ug/ml lipopolysaccharide (LPS) for 2 hours, whole cell lysate

Lysates/proteins at 20 µg per lane.

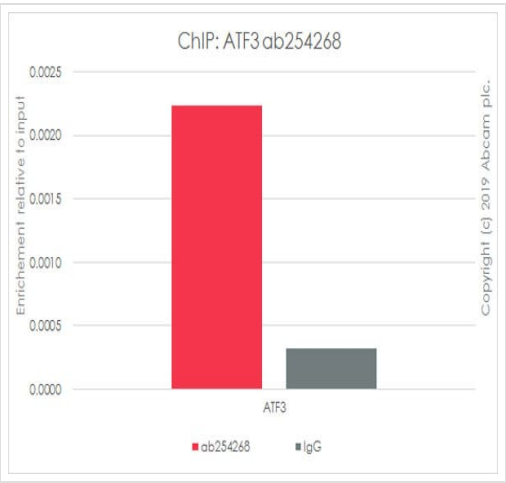
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 70 seconds

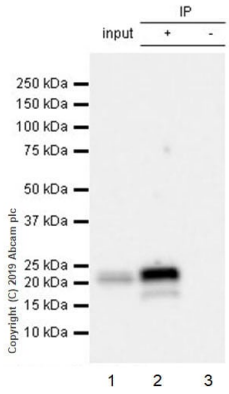
Blocking and dilution buffer: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID: 24973221; 24062788).



ChIP - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

Chromatin was prepared from HeLa cells according to the Abcam Dual X-ChIP protocol. Cells were fixed with EGS for 30min, then formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 5 µg of ab254268 (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.



Immunoprecipitation - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

ATF3 was immunoprecipitated from 0.35 mg RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 1ug/ml lipopolysaccharide (LPS) for 2h whole cell lysate 10ug with ab254268 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab254268 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/1000 dilution.

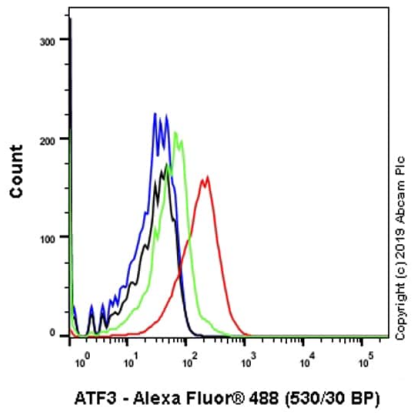
Lane 1: RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 1ug/ml lipopolysaccharide (LPS) for 2h whole cell lysate 10ug.

Lane 2: ab254268 IP in RAW264.7 treated with 1ug/ml lipopolysaccharide (LPS) for 2h whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab254268 in RAW264.7 treated with 1ug/ml lipopolysaccharide (LPS) for 2h whole cell lysate.

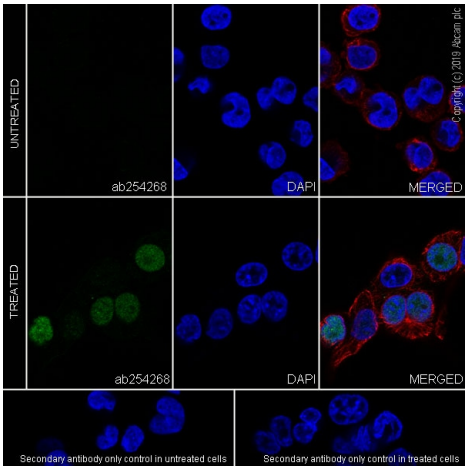
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 6 seconds.



Flow Cytometry (Intracellular) - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

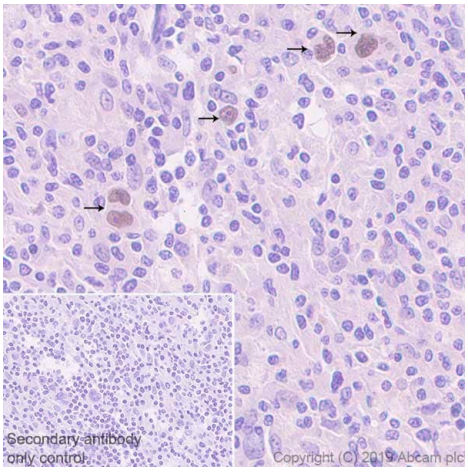
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized THP-1 (human monocytic leukemia monocyte) treated with 80nM Phorbol 12-myristate 13-acetate (PMA) for 16h, then together with 1µg/ml lipopolysaccharides (LPS) for 8h (Red) / Untreated control (Green) cells labelling ATF3 with ab254268 at 1/600 compared with a Rabbit monoclonal IgG (**ab172730**) isotype control (Black) 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.



Immunocytochemistry/ Immunofluorescence - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized THP-1 (human monocytic leukemia monocyte) cells labelling ATF3 with ab254268 at 1/100 (5.7 ug/ml) dilution, followed by **ab150077** AlexaFluor® 488 Goat anti-Rabbit secondary antibody at 1/1000 (5.7 ug/ml) dilution (Green). Confocal image showing nuclear staining in THP-1 cells treated with Phorbol 12-myristate 13-acetate (80 nM) for 16 h, then along with lipopolysaccharides (1ug/ml) for 8 h. **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

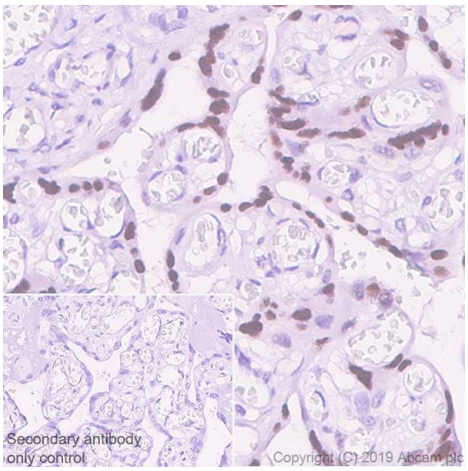
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab150077** AlexaFluor® 488 Goat anti-Rabbit secondary at 1/1000 (2 ug/ml) dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

Immunohistochemical analysis of paraffin-embedded Human tissue labeling ATF3 with ab254268 at 1/500 dilution (1.14 ug/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining in Reed-Sternberg (HRS) cells of human (PMID: 16263788) is observed. Counterstained with Hematoxylin. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

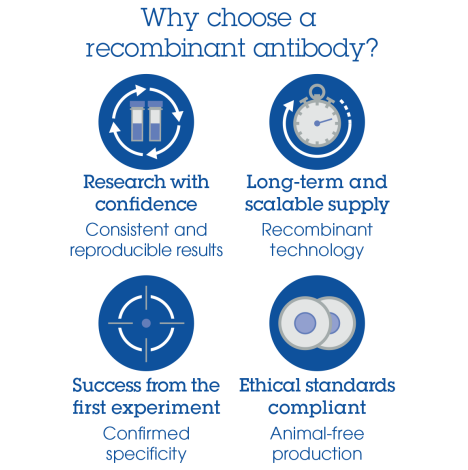
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

Immunohistochemical analysis of paraffin-embedded Human placenta tissue labeling ATF3 with ab254268 at 1/500 dilution (1.14 ug/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining in human placenta (PMID/ 28947613). Counterstained with Hematoxylin. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

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



Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268)

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