abcam

Product datasheet

Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free ab201809

KO 評価済 リコンビナント RabMAb

1 Abreviews 2 References 画像数 10

製品の概要

製品名	Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free
製品の詳細	Rabbit monoclonal [EPR2418Y] to IRF3 - BSA and Azide free
由来種	Rabbit
アプリケーション	**適用あり:** IHC-P, WB, ICC/IF, Flow Cyt (Intra) **適用なし:** IP
種交差性	**交差種:** Mouse, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: A549, HeLa, MCF7, Jurkat, THP-1, Daudi, HepG2 whole cell lysates. Human fetal heart and kidney lysates. Mouse heart and spleen lysates, NIH/3T3 whole cell lysates. IHC-P: Human tonsil, Human squamous cell carcinoma of cervix, Mouse spleen. ICC/IF: HeLa cells Flow: U937 cells
特記事項	ab201809 is the carrier-free version of **ab68481**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.2 Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR2418Y
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ab201809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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 51 kDa (predicted molecular weight: 47 kDa).
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

追加情報 Is unsuitable for IP.

ターゲット情報

機能 Mediates interferon-stimulated response element (ISRE) promoter activation. Functions as a molecular switch for antiviral activity. DsRNA generated during the course of an viral infection leads to IRF3 phosphorylation on the C-terminal serine/threonine cluster. This induces a conformational change, leading to its dimerization, nuclear localization and association with CREB binding protein (CREBBP) to form dsRNA-activated factor 1 (DRAF1), a complex which activates the transcription of genes under the control of ISRE. The complex binds to the IE and PRDIII regions on the IFN-alpha and IFN-beta promoters respectively. IRF-3 does not have any transcription activation domains.

組織特異性 Expressed constitutively in a variety of tissues.

配列類似性 Belongs to the IRF family.
Contains 1 IRF tryptophan pentad repeat DNA-binding domain.

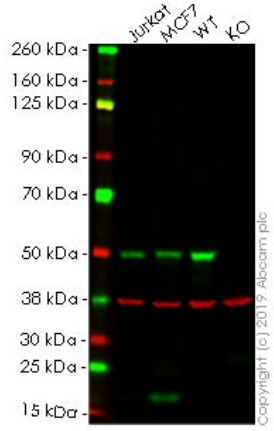
翻訳後修飾 Constitutively phosphorylated on many serines residues. C-terminal serine/threonine cluster is phosphorylated in response of induction by IKBKE and TBK1. Ser-385 and Ser-386 may be

specifically phosphorylated in response to induction. An alternate model propose that the five serine/threonine residues between 396 and 405 are phosphorylated in response to a viral infection. Phosphorylation, and subsequent activation of IRF3 is inhibited by vaccinia virus protein E3.
Ubiquitinated; ubiquitination involves RBCK1 leading to proteasomal degradation. Polyubiquitinated; ubiquitination involves TRIM21 leading to proteasomal degradation. ISGylated by HERC5 resulting in sustained IRF3 activation and in the inhibition of IRF3 ubiquitination by disrupting PIN1 binding. The phosphorylation state of IRF3 does not alter ISGylation.

細胞内局在

Cytoplasm. Nucleus. Shuttles between cytoplasmic and nuclear compartments, with export being the prevailing effect. When activated, IRF3 interaction with CREBBP prevents its export to the cytoplasm.

画像



Western blot - Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

All lanes : Anti-IRF3 antibody [EPR2418Y] (**ab68481**) at 1/1000 dilution

Lane 1 : Jurkat cell lysate
Lane 2 : MCF7 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : IRF3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

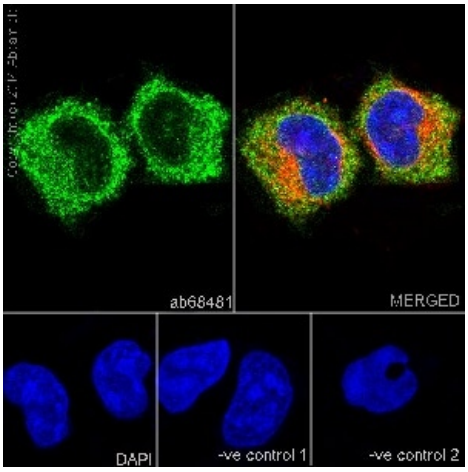
Predicted band size: 47 kDa
Observed band size: 51 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab68481**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab68481** observed at 50 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab68481 was shown to react with IRF3 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab255345** (knockout cell lysate **ab263784**) was used. Wild-type and IRF3 knockout samples were subjected to SDS-PAGE. **ab68481** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit

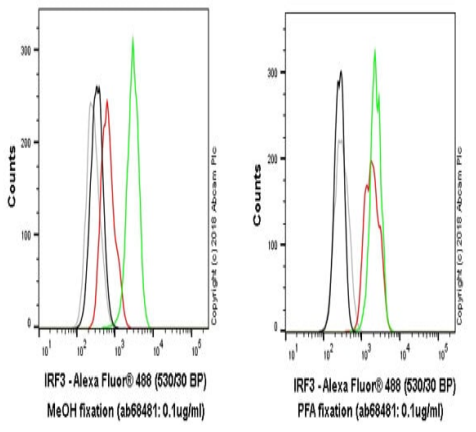
IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling IRF3 with **ab68481** at 1/100 dilution. Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/400 dilution was used as the secondary antibody (green). The confocal image shows cytoplasmic on HeLa cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows;
1. **ab68481** at 1/100 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab68481**).



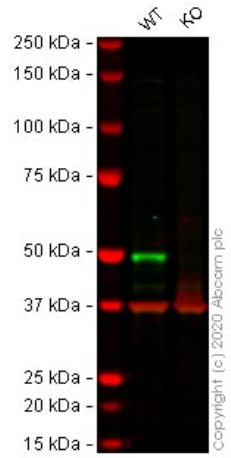
Flow Cytometry (Intracellular) - Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-IRF3 knockout cells (red line) stained with **ab68481**. The cells were fixed with 80% methanol (5 min) (left pannel) or 4% formaldehyde (10 min) (right pannel), and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (**ab68481**, 0.1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-IRF3 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

Note: We recommend fixing cells using MeOH instead of PFA

to get optimal results.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab68481**).



Western blot - Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

All lanes : Anti-IRF3 antibody [EPR2418Y] (**ab68481**) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : IRF3 knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

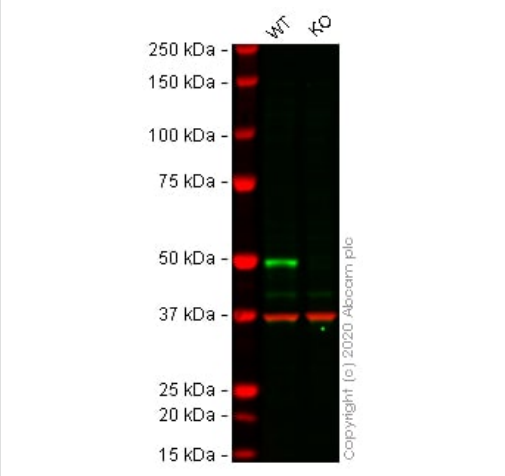
Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 50 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab68481**).

Lanes 1 - 2: Merged signal (red and green). Green - **ab68481** observed at 50 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab68481 was shown to react with IRF3 in wild-type A549 cells in western blot with loss of signal observed in IRF3 knockout cell line **ab267098** (IRF3 knockout cell lysate **ab256954**). Wild-type and IRF3 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab68481** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

All lanes : Anti-IRF3 antibody [EPR2418Y] (**ab68481**) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : IRF3 knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

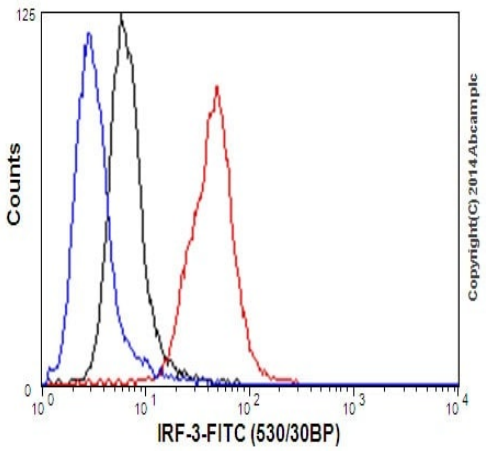
Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 50 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab68481**).

Lanes 1 - 2: Merged signal (red and green). Green - **ab68481** observed at 50 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

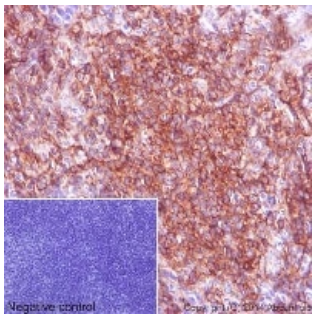
ab68481 was shown to react with IRF3 in wild-type A549 cells in western blot with loss of signal observed in IRF3 knockout cell line **ab267097** (IRF3 knockout cell lysate **ab256953**). Wild-type and IRF3 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab68481** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

Intracellular Flow Cytometry analysis of 2% paraformaldehyde fixed U937 (Human histiocytic lymphoma cells) cells labeling IRF3 with **ab68481** at 1/160 dilution (red line). Secondary antibody used is a goat anti rabbit IgG (FITC) at 1/150 dilution. The isotype control is rabbit monoclonal IgG (black line). The unlabeled control is cells without incubation with primary and secondary antibodies (blue line).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab68481**).

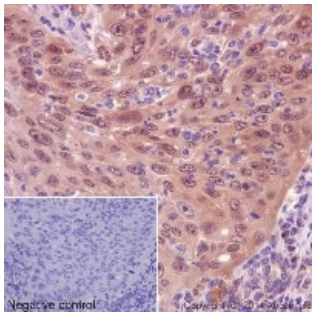


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

Immunohistochemical analysis of paraffin-embedded Mouse spleen labeling IRF3 with **ab68481**at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab68481**).

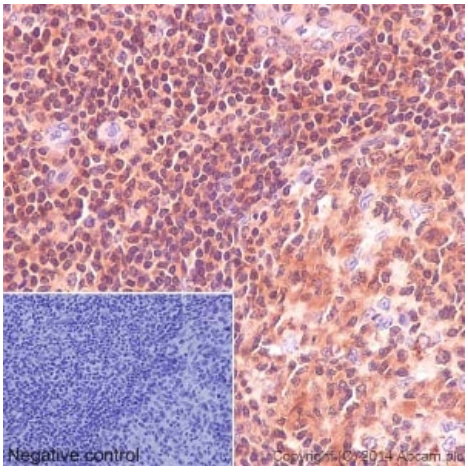


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

Immunohistochemical analysis of paraffin-embedded Human squamous cell carcinoma of cervix labeling IRF3 with **ab68481**at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab68481**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

Immunohistochemical analysis of paraffin-embedded Human tonsil labeling IRF3 with **ab68481**at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab68481**).

Anti-IRF3 antibody [EPR2418Y] - BSA and Azide free (ab201809)

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