

Product datasheet

Anti-IRF3 antibody [EPR2418Y] ab68481

KO VALIDATED Recombinant RabMAB

★★★★★ 2 Abreviews 34 References 15 Images

Overview

Product name	Anti-IRF3 antibody [EPR2418Y]
Description	Rabbit monoclonal [EPR2418Y] to IRF3
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IHC-P Unsuitable for: IP
Species reactivity	Reacts with: Mouse, Human
Immunogen	Synthetic peptide within Human IRF3 aa 50-150. The exact sequence is proprietary. Database link: Q14653 (Peptide available as ab203561)
Positive control	WB: A549, HeLa, 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, HAP1-wt cells.
General notes	Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. 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: 40% Glycerol, 0.05% BSA, 59% PBS

Purity Protein A purified
Clonality Monoclonal
Clone number EPR2418Y
Isotype IgG

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab68481 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
Flow Cyt (Intra)		1/160. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. MeOH fixation is recommended.
ICC/IF		1/100.
WB	★★★★★ (1)	1/1000. Detects a band of approximately 51 kDa (predicted molecular weight: 47 kDa). Can be blocked with IRF3 peptide (ab203561) .
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for IP.

Target

Function Mediates interferon-stimulated response element (ISRE) promoter activation. Functions as a molecular switch for antiviral activity. DsRNA generated during the course of a 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.

Tissue specificity Expressed constitutively in a variety of tissues.

Sequence similarities Belongs to the IRF family.
Contains 1 IRF tryptophan pentad repeat DNA-binding domain.

Post-translational modifications Constitutively phosphorylated on many serines residues. C-terminal serine/threonine cluster is phosphorylated in response of induction by IKKε 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.

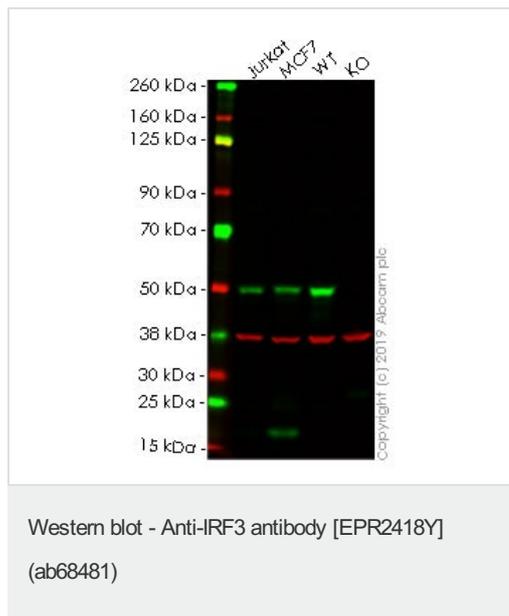
Polyubiquitinated; ubiquitination involves TRIM21 leading to proteasomal degradation.

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.

Cellular localization

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.

Images



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

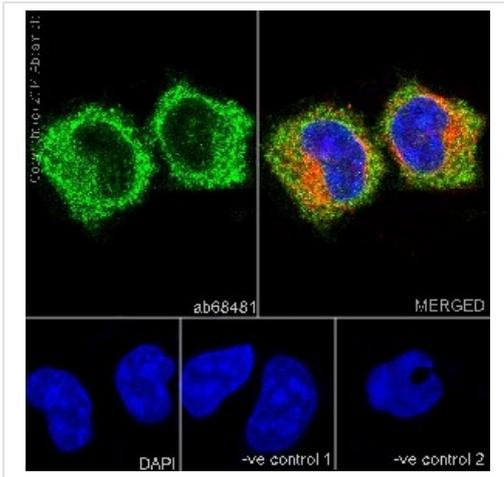
Lane 4 : IRF3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 47 kDa

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.

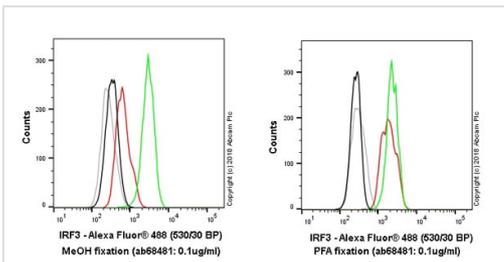


Immunocytochemistry/ Immunofluorescence - Anti-IRF3 antibody [EPR2418Y] (ab68481)

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.



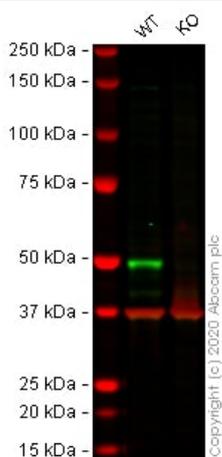
Flow Cytometry (Intracellular) - Anti-IRF3 antibody [EPR2418Y] (ab68481)

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 panel) or 4% formaldehyde (10 min) (right panel), 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.



Western blot - Anti-IRF3 antibody [EPR2418Y] (ab68481)

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.

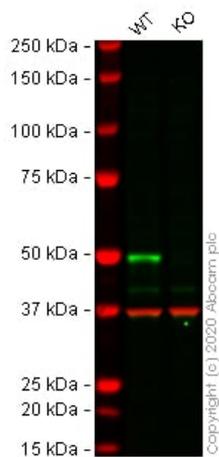
Performed under reducing conditions.

Predicted band size: 47 kDa

Observed band size: 50 kDa

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] (ab68481)

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.

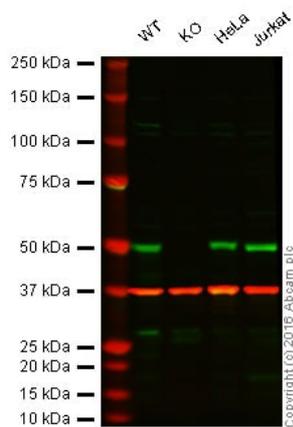
Performed under reducing conditions.

Predicted band size: 47 kDa

Observed band size: 50 kDa

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.



Western blot - Anti-IRF3 antibody [EPR2418Y] (ab68481)

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

Lane 1 : Wild-type HAP1 cell lysate

Lane 2 : IRF3 knockout HAP1 cell lysate

Lane 3 : HeLa cell lysate

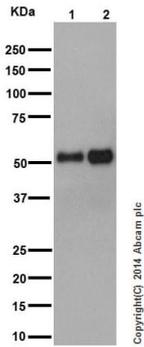
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 47 kDa

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 HAP1 cells along with additional cross-reactive bands. No band was observed when IRF3 knockout samples were examined. Wild-type and IRF3 knockout samples were subjected to SDS-PAGE. ab68481 and [ab8245](#) (loading control to GAPDH) were both diluted to 1/1000 and 1/10,000 respectively and incubated overnight at 4°C. 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 1/10,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-IRF3 antibody [EPR2418Y] (ab68481)

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

Lane 1 : Human fetal heart lysate

Lane 2 : Human fetal kidney lysate

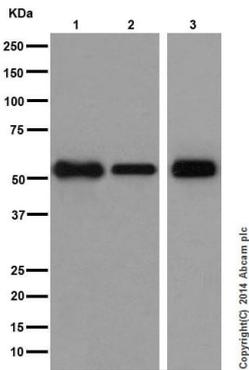
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 47 kDa

Blocking and Dilution buffer: 5% NFDM/TBST



Western blot - Anti-IRF3 antibody [EPR2418Y] (ab68481)

All lanes : Anti-IRF3 antibody [EPR2418Y] (ab68481) at 1/10000 dilution

Lane 1 : THP-1 (Human monocytic leukemia cells) whole cell lysates

Lane 2 : HepG2 (Human liver hepatocellular carcinoma) whole cell lysates

Lane 3 : Daudi (Human Burkitt's lymphoma cell line) whole cell lysates

Lysates/proteins at 10 µg per lane.

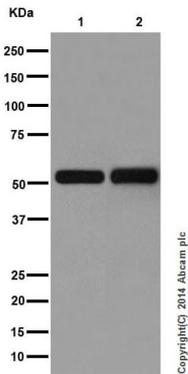
Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 47 kDa

Observed band size: 51 kDa

Blocking and Dilution buffer: 5% NFDM/TBST



Western blot - Anti-IRF3 antibody [EPR2418Y] (ab68481)

All lanes : Anti-IRF3 antibody [EPR2418Y] (ab68481) at 1/10000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates

Lane 2 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysates

Lysates/proteins at 20 µg per lane.

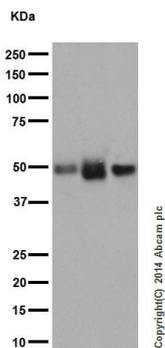
Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 47 kDa

Observed band size: 51 kDa

Blocking and Dilution buffer: 5% NFDM/TBST



Western blot - Anti-IRF3 antibody [EPR2418Y] (ab68481)

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

Lane 1 : Mouse heart lysate

Lane 2 : Mouse spleen lysate

Lane 3 : NIH/3T3 (Mouse embryo fibroblast cells) whole cell lysates

Lysates/proteins at 10 µg per lane.

Secondary

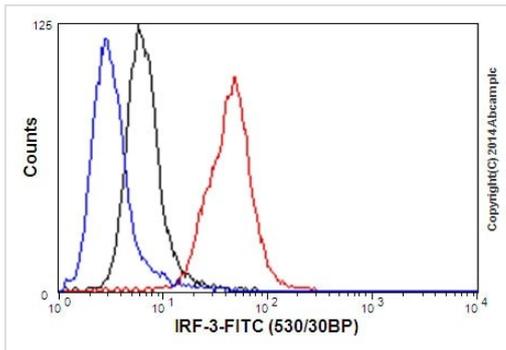
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 47 kDa

Observed band size: 47 kDa

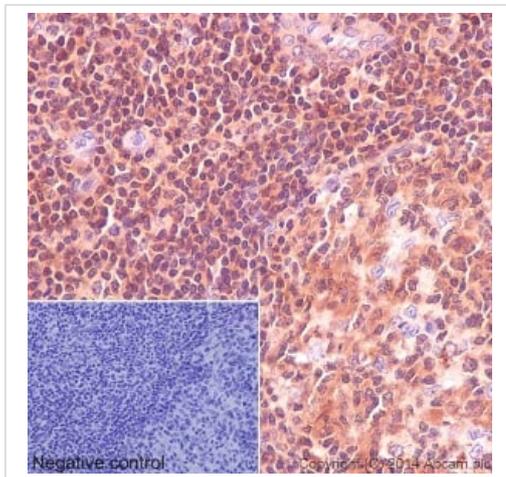
Blocking and Dilution buffer: 5% NFDM/TBST

The slightly smaller molecular mass observed in mouse than in human is supported by literature.



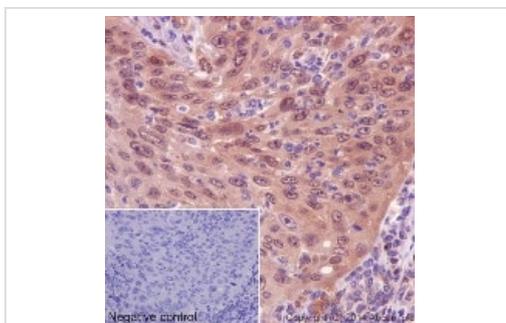
Flow Cytometry (Intracellular) - Anti-IRF3 antibody [EPR2418Y] (ab68481)

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).



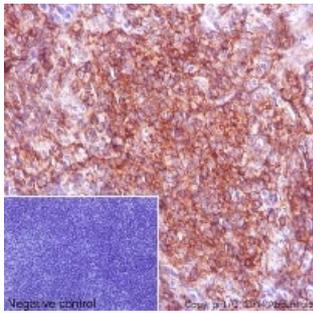
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRF3 antibody [EPR2418Y] (ab68481)

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRF3 antibody [EPR2418Y] (ab68481)

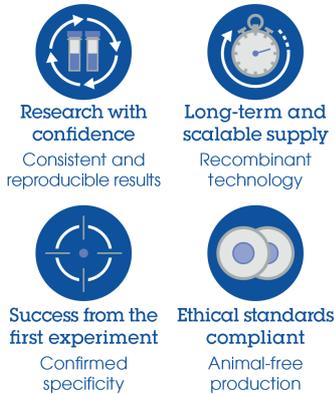
Immunohistochemical analysis of paraffin-embedded Human squamous cell carcinoma of cervix labeling IRF3 with ab68481 at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRF3 antibody [EPR2418Y] (ab68481)

Why choose a recombinant antibody?



Anti-IRF3 antibody [EPR2418Y] (ab68481)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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