abcam

ab76409

Anti-IRF3 antibody [EP2419Y]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP2419Y
Purification technique	Affinity purification Protein A
Concentration	0.143 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	Perform heat-mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

ICC/IF

Not recommended

Species	Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/20
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/2000
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information IRF3

Function	Key transcriptional regulator of type I interferon (IFN)-dependent immune responses which plays a critical role in the innate immune response against DNA

and RNA viruses (PubMed:22394562, PubMed:24049179, PubMed:25636800, PubMed:27302953, PubMed:31340999, PubMed:36603579, PubMed:8524823, PubMed:39362857). Regulates the transcription of type I IFN genes (IFN-alpha and IFN-beta) and IFN-stimulated genes (ISG) by binding to an interferon-stimulated response element (ISRE) in their promoters (PubMed:11846977, PubMed:16846591, PubMed:16979567, PubMed:20049431, PubMed:32972995, PubMed:36603579, PubMed:8524823). Acts as a more potent activator of the IFN-beta (IFNB) gene than the IFN-alpha (IFNA) gene and plays a critical role in both the early and late phases of the IFNA/B gene induction (PubMed:16846591, PubMed:16979567, PubMed:20049431, PubMed:36603579). Found in an inactive form in the cytoplasm of uninfected cells and following viral infection, double-stranded RNA (dsRNA), or toll-like receptor (TLR) signaling, is phosphorylated by IKBKE and TBK1 kinases (PubMed:22394562, PubMed:25636800, PubMed:27302953, PubMed:36603579). This induces a conformational change, leading to its dimerization and nuclear localization and association with CREB binding protein (CREBBP) to form dsRNA-activated factor 1 (DRAF1), a complex which activates the transcription of the type I IFN and ISG genes (PubMed:16154084, PubMed:27302953, PubMed:33440148, PubMed:36603579). Can activate distinct gene expression programs in macrophages and can induce significant apoptosis in primary macrophages (PubMed:16846591). In response to Sendai virus infection, is recruited by TOMM70:HSP90AA1 to mitochondrion and forms an apoptosis complex TOMM70:HSP90AA1:IRF3:BAX inducing apoptosis (PubMed:25609812). Key transcription factor regulating the IFN response during SARS-CoV-2 infection (PubMed:33440148).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	IRF3 also known as Interferon Regulatory Factor 3 acts as an important transcription factor in the immune response. It has a molecular weight of approximately 47 kDa. The IRF3 protein is mainly expressed in the cytoplasm and nucleus of various cell types including immune cells such as macrophages and dendritic cells. The protein becomes activated through phosphorylation a process frequently identified in its phosphorylated form phospo-IRF3 or p-IRF3 which facilitates its role in immune function.
Biological function summary	IRF3 participates in the regulation of type I interferon (IFN) response a fundamental antiviral defense mechanism. IRF3 when phosphorylated forms a complex with CBP/p300 which then translocates to the nucleus to drive the expression of IFN-stimulated genes. This action strengthens the innate immune response and boosts the body's ability to counteract viral infections. Its activity and regulation are significant for maintaining a balanced immune response without excessive inflammation.
Pathways	IRF3 is involved in the Toll-like receptor (TLR) and RIG-I-like receptor (RLR) signaling pathways both essential in pathogen recognition and response. Within these pathways IRF3 interacts with proteins such as MAVS and TBK1 to propagate immune signaling. The activation of IRF3 in these pathways results in the production of type I interferons and other cytokines orchestrating an effective antiviral response. These interactions highlight the protein's central role in mediating immune signaling cascades.
Associated diseases and disorders	IRF3's malfunction or deregulation can contribute to autoimmune diseases and antiviral deficiencies. Conditions such as systemic lupus erythematosus (SLE) and chronic hepatitis B infection are linked to IRF3 activity. During autoimmune responses or viral persistence the aberrant activation of IRF3 can lead to inappropriate immune responses. The connection with proteins like STAT1 in these conditions highlights the complex network IRF3 engages in facilitating its impact on disease progression and immune dysregulation.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

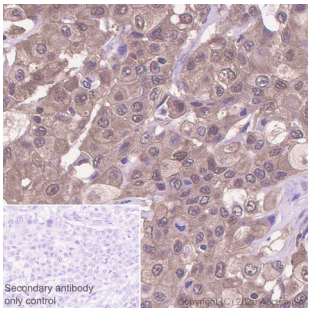
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

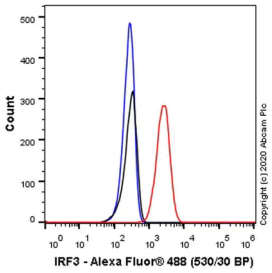
7 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRF3 antibody [EP2419Y] (ab76409)

IRF3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IRF3 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human bladder carcinoma tissue sections labeling IRF3 with Purified 76409 at 1:100 dilution (1.55 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

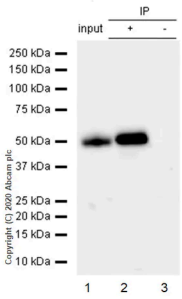


Flow Cytometry (Intracellular) - Anti-IRF3 antibody [EP2419Y] (ab76409)

IRF3 Flow Cytometry (Intracellular) staining using rabbit Anti-IRF3 antibody

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling IRF3 with Purified 76409 at 1/20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

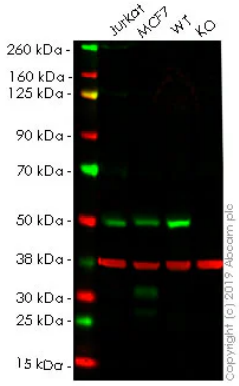
Immunoprecipitation - Anti-IRF3 antibody [EP2419Y] (ab76409)



Purified ab76409 at 1/20 dilution (0.8µg) immunoprecipitating IRF3 in HeLa whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab76409 + HeLa whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab76409 in HeLa whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 47 kDa
Fresh lysate need to be used to avoid protein degradation.
All lanes:
Immunoprecipitation - Anti-IRF3 antibody [EP2419Y] (ab76409)

Predicted band size: 47 kDa

Western blot - Anti-IRF3 antibody [EP2419Y] (ab76409)



IRF3 Western blot staining using rabbit Anti-IRF3 antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab76409 observed at 50 kDa. Red - loading control, ab8245 observed at 37 kDa.
ab76409 was shown to react with IRF3 in wild-type HeLa. 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. ab76409 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.

All lanes:
Western blot - Anti-IRF3 antibody [EP2419Y] (ab76409) at 1/1000 dilution

Lane 1:
Jurkat cell lysate at 20 µg

Lane 2:
MCF7 cell lysate at 20 µg

Lane 3:
Wild-type HeLa cell lysate at 20 µg

Lane 4:
IRF3 knockout HeLa cell lysate at 20 µg

Lane 4:
Western blot - Human IRF3 knockout HeLa cell lysate (ab263784) at 20 µg

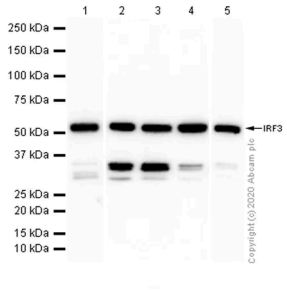
Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 47 kDa

Observed band size: 47 kDa, 50 kDa



Western blot - Anti-IRF3 antibody [EP2419Y] (ab76409)

We are unsure how to define the extra bands.
All lanes:
Western blot - Anti-IRF3 antibody [EP2419Y] (ab76409) at 1/1000 dilution

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

Lane 3:
U-937 (Human histiocytic lymphoma monocyte) whole cell lysate at 20 µg

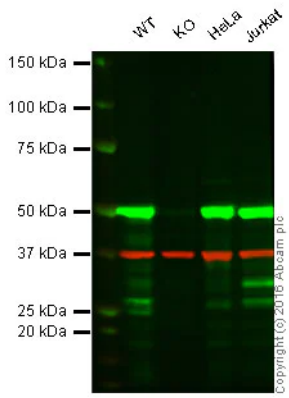
Lane 4:
THP-1 (Human monocytic leukemia monocyte) whole cell lysate at 20 µg

Lane 5:
Daudi (Human Burkitt's lymphoma lymphoblast) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 47 kDa



Western blot - Anti-IRF3 antibody [EP2419Y] (ab76409)

Lanes 1 - 4: Merged signal (red and green). Green - ab76409 observed at 50 kDa. Red - loading control, ab8245, observed at 37kDa.

ab76409 was shown to react with IRF3 in wild-type HAP1 cells alond with additional cross-reactive bands. No band was observed when IRF3 knockout samples were used. Wild-type and IRF3 knockout samples were subjected to SDS-PAGE. ab76409 and ab8245 (loading control to GAPDH) were both diluted 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.

All lanes:
Western blot - Anti-IRF3 antibody [EP2419Y] (ab76409) at 1/1000 dilution

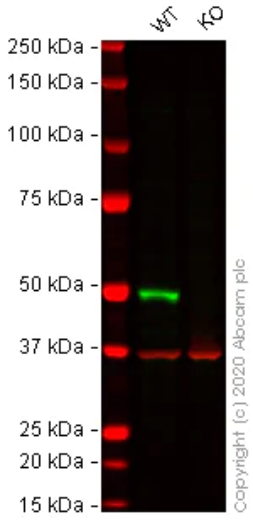
Lane 1:
Wild-type HAP1 cell lysate at 20 µg

Lane 2:
IRF3 knockout HAP1 cell lysate at 20 µg

Lane 3:
HeLa cell lysate at 20 µg

Lane 4:
Jurkat cell lysate at 20 µg

Predicted band size: 47 kDa



Western blot - Anti-IRF3 antibody [EP2419Y] (ab76409)

IRF3 Western blot staining using rabbit Anti-IRF3 antibody

Lanes 1 - 2: Merged signal (red and green). Green - ab76409 observed at 50 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab76409 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 ab76409 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.

All lanes:
Western blot - Anti-IRF3 antibody [EP2419Y] (ab76409) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
IRF3 knockout A549 cell lysate at 20 µg

Lane 2:
Western blot - Human IRF3 knockout A549 cell line (ab267097)

Performed under reducing conditions.

Predicted band size: 47 kDa

Observed band size: 50 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.