abcam

Product datasheet

Anti-IRF3 antibody [EP2419Y] ab76409

敲除验证 重组 RabMAb

22 References 7 图像

概述

产品名称	Anti-IRF3抗体[EP2419Y]
描述	兔单克隆抗体[EP2419Y] to IRF3
宿主	Rabbit
经测试应用	**适用于:** WB, IP, IHC-P, Flow Cyt (Intra) **不适用于:** ICC/IF
种属反应性	**与反应:** Human
免疫原	Synthetic peptide within Human IRF3 (C terminal). The exact sequence is proprietary.
阳性对照	WB: A549, MCF7, HeLa, HAP1, U-937, THP-1,Daudi and Jurkat cell lysates. IP: HeLa whole cell lysate. IHC: Human bladder carcinoma tissue. Flow Cyt (intra): HeLa cells.
常规说明	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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
纯度	Protein A purified
克隆	单克隆

克隆编号	EP2419Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab76409于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		1/1000 - 1/2000. Predicted molecular weight: 47 kDa.
IP		1/20.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

应用说明 Is unsuitable for ICC/IF.

靶标

功能 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.

组织特异性 Expressed constitutively in a variety of tissues.

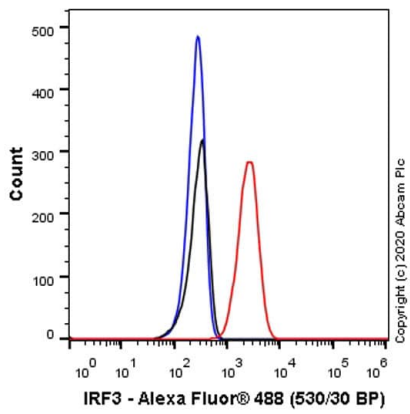
序列相似性 Belongs to the IRF family.
Contains 1 IRF tryptophan pentad repeat DNA-binding domain.

翻译后修饰 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.

细胞定位 Cytoplasm. Nucleus. Shuttles between cytoplasmic and nuclear compartments, with export being

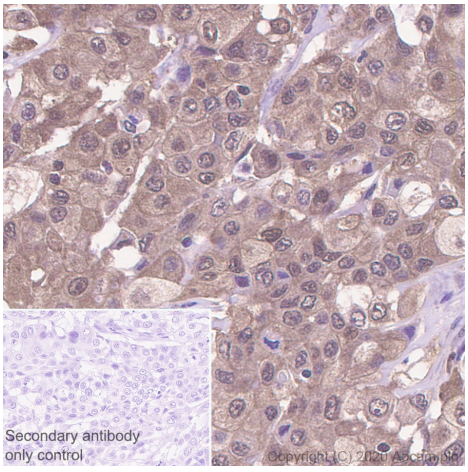
the prevailing effect. When activated, IRF3 interaction with CREBBP prevents its export to the cytoplasm.

图片



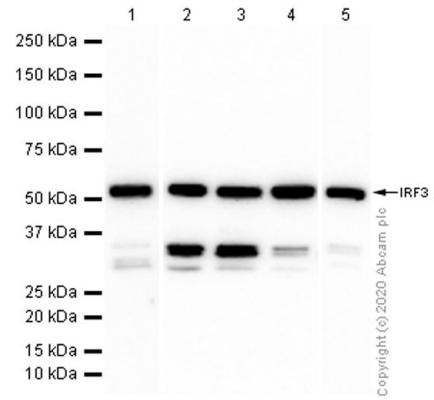
Flow Cytometry (Intracellular) - Anti-IRF3 antibody [EP2419Y] (ab76409)

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).



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

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.



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

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

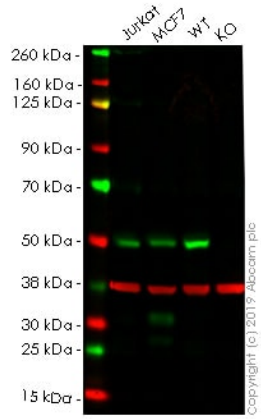
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate
Lane 3 : U-937 (Human histiocytic lymphoma monocyte) whole cell lysate
Lane 4 : THP-1 (Human monocytic leukemia monocyte) whole cell lysate
Lane 5 : Daudi (Human Burkitt's lymphoma lymphoblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 47 kDa

We are unsure how to define the extra bands.



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

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

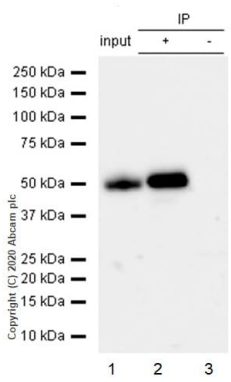
Lane 1 : Jurkat cell lysate
Lane 2 : MCF7 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : IRF3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 47 kDa

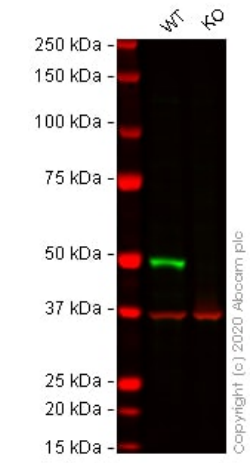
Lanes 1 - 4: Merged signal (red and green). Green - ab76409 observed at 50 kDa. Red - loading control, **ab8245** observed at 37 kDa.

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.



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.



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

All lanes : Anti-IRF3 antibody [EP2419Y] (ab76409) 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.

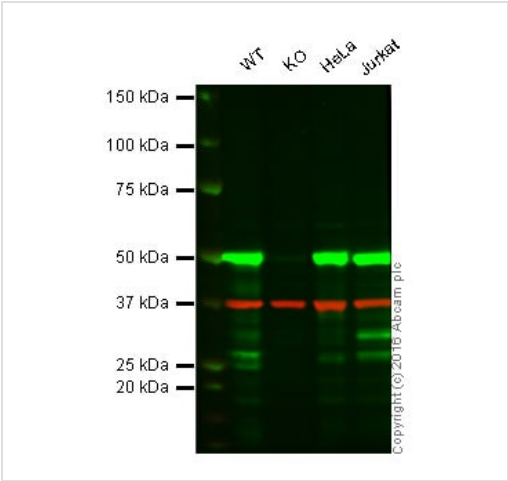
Performed under reducing conditions.

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

Lanes 1 - 2: Merged signal (red and green). Green - 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.



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

All lanes : Anti-IRF3 antibody [EP2419Y] (ab76409) 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 - 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.

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