abcam

Product datasheet

Anti-IFNGR1 antibody [EPR7866] - BSA and Azide free ab226151



Recombinant

RabMAb

8 Images

Overview

Product name Anti-IFNGR1 antibody [EPR7866] - BSA and Azide free

Description Rabbit monoclonal [EPR7866] to IFNGR1 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, Flow Cyt (Intra), ICC/IF

Unsuitable for: IP

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HEK-293, HeLa and HepG2 cell lysates. IHC-P: Human tonsil tissue. Flow Cyt (intra): HeLa

cells, HEK293 cells. ICC/IF: MCF7 cells

General notes ab226151 is the carrier-free version of <u>ab134070</u>.

Our <u>carrier-free</u> 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 <u>conjugation kits</u> 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.

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

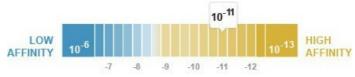
monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Dissociation constant (K_D) $K_D = 1.20 \times 10^{-11} M$



Learn more about K_D

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clone number Monoclonal EPR7866

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab226151 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
WB		Use at an assay dependent concentration. Detects a band of approximately 90 kDa (predicted molecular weight: 54 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

Function Receptor for interferon gamma. Two receptors bind one interferon gamma dimer.

Involvement in disease Defects in IFNGR1 are a cause of mendelian susceptibility to mycobacterial disease (MSMD)

[MIM:209950]; also known as familial disseminated atypical mycobacterial infection. This rare condition confers predisposition to illness caused by moderately virulent mycobacterial species, such as Bacillus Calmette-Guerin (BCG) vaccine and environmental non-tuberculous mycobacteria, and by the more virulent Mycobacterium tuberculosis. Other microorganisms rarely cause severe clinical disease in individuals with susceptibility to mycobacterial infections, with the exception of Salmonella which infects less than 50% of these individuals. The pathogenic mechanism underlying MSMD is the impairment of interferon-gamma mediated immunity whose severity determines the clinical outcome. Some patients die of overwhelming mycobacterial disease with lepromatous-like lesions in early childhood, whereas others develop, later in life, disseminated but curable infections with tuberculoid granulomas. MSMD is a genetically heterogeneous disease with autosomal recessive, autosomal dominant or X-linked inheritance.

Sequence similarities

Belongs to the type II cytokine receptor family.

Contains 2 fibronectin type-III domains.

Contains 2 lg-like C2-type (immunoglobulin-like) domains.

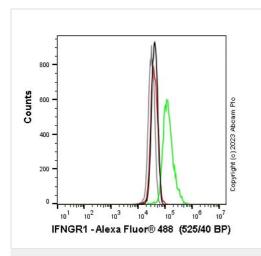
Post-translational modifications

Phosphorylated at Ser/Thr residues.

Cellular localization

Membrane.

Images



Flow Cytometry (Intracellular) - Anti-IFNGR1 antibody [EPR7866] - BSA and Azide free (ab226151)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134070).

Flow cytometry overlay histogram showing wild-type HEK293 (green line) and IFNGR1 knockout HEK293 stained with <u>ab134070</u> (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (<u>ab134070</u>) (1x 10^6 in 100μ I at $0.2~\mu$ g/mI (1/10000)) for 30min at 22° C.

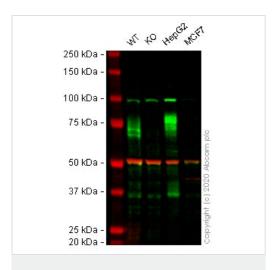
The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type HEK293 - black line, IFNGR1 knockout HEK293 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in HEK293 Fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for

15 min under the same conditions.



Western blot - Anti-IFNGR1 antibody [EPR7866] - BSA and Azide free (ab226151)

All lanes : Anti-IFNGR1 antibody [EPR7866] (ab134070) at 1/1000 dilution

Lane 1 : Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 2: IFNGR1 knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 3: Hep G2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

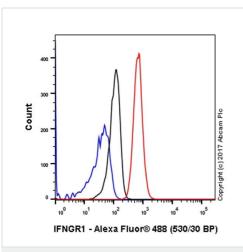
Predicted band size: 54 kDa

Observed band size: 60-80 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab134070</u>).

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab134070</u> observed at 60-80 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

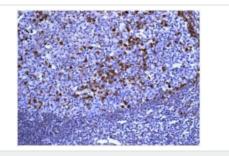
ab134070 was shown to react with IFNGR1 in wild-type HEK-293 cells in western blot with loss of signal observed in IFNGR1 knockout sample. Wild-type and IFNGR1 knockout HEK-293 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab134070 and ab7291 (Mouse anti-Alpha Tubulin [DM1A] 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-IFNGR1 antibody [EPR7866] - BSA and Azide free (ab226151)

This Flow Cyt data was generated using the same anti-IFNGR1 antibody clone, EPR7866, in a different buffer formulation (cat **ab134070**).

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling IFNGR1 (red) with **ab134070** at a 1/1000 dilution. Cells were fixed with 80% methanol and permeabilized with 0.1% Tween-20. A goat anti-rabbit IgG (Alexa Fluor[®]488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (**ab172730**). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.



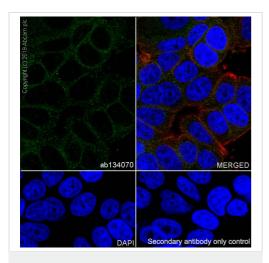
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IFNGR1 antibody

[EPR7866] - BSA and Azide free (ab226151)

This IHC data was generated using the same anti-IFNGR1 antibody clone, EPR7866, in a different buffer formulation (cat# **ab134070**).

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labelling IFNGR1 with <u>ab134070</u> at 1/100 dilution.

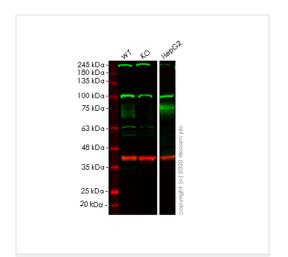
Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-IFNGR1 antibody [EPR7866] - BSA and Azide free (ab226151)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (human breast adenocarcinoma epithelial cell) cells labeling IFNGR1 with purified <u>ab134070</u> at 1/100 dilution (10 μg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) 1/200 (2.5 μg/mL). Goat anti rabbit lgG (Alexa Fluor[®] 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2 μg/mL) dilution. DAPI (blue) was used as nuclear counterstain. <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) 1/200 (2.5 μg/mL) was used as the secondary antibody only control.

This data was generated using the same anti-IFNGR1 antibody clone, EPR7866, in a different buffer formulation (cat# <u>ab134070</u>).



Western blot - Anti-IFNGR1 antibody [EPR7866] - BSA and Azide free (ab226151)

All lanes : Anti-IFNGR1 antibody [EPR7866] (ab134070) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: IFNGR1 knockout HeLa cell lysate

Lane 3: HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

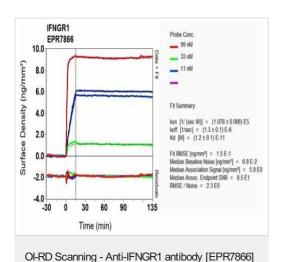
Predicted band size: 54 kDa **Observed band size:** 70-95 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab134070</u>).

Lanes 1-3: Merged signal (red and green). Green - <u>ab134070</u> observed at 70-95 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

<u>ab134070</u> Anti-IFNGR1 antibody [EPR7866] was shown to specifically react with IFNGR1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line <u>ab265111</u> (knockout cell lysate <u>ab257477</u>) was used. Wild-type and IFNGR1 knockout

samples were subjected to SDS-PAGE. <u>ab134070</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) 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 (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

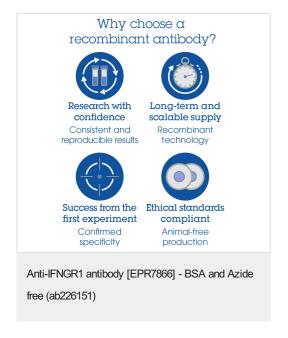


- BSA and Azide free (ab226151)

Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134070).



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