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Anti-IRF3 Antibody Picoband™ (monoclonal, 3B4)

Catalog # ABO15032

Product Information

Application	WB, IF, ICC, FC
Primary Accession	P70671
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Rat, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-IRF3 Antibody Picoband™ (monoclonal, 3B4) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	54131
Other Names	Interferon regulatory factor 3, IRF-3, Irf3
Calculated MW	46852
Application Details	Western blot, 0.25-0.5 □g/ml, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5 □g/ml, Mouse Flow Cytometry, 1-3 □g/1x10^6 cells, Mouse
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 3B4
Immunogen	E.coli-derived mouse IRF3 recombinant protein (Position: M1-I419).
Purification	Immunogen affinity purified.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	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:15800576, PubMed:39719712). 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:15800576, PubMed:39719712). 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). 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:16846591, PubMed:16979567, PubMed:20049431). 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:16846591, PubMed:16979567, PubMed:20049431). Can activate distinct gene expression programs in macrophages and can induce significant apoptosis in primary macrophages (PubMed:16846591, PubMed:16979567, PubMed:20049431).

Cellular Location

Cytoplasm. Nucleus {ECO:0000250|UniProtKB:Q14653}. Mitochondrion {ECO:0000250|UniProtKB:Q14653}. Note=Shuttles between cytoplasmic and nuclear compartments, with export being the prevailing effect. When activated, IRF3 interaction with CREBBP prevents its export to the cytoplasm. Recruited to mitochondria via TOMM70:HSP90AA1 upon Sendai virus infection. {ECO:0000250|UniProtKB:Q14653}

Tissue Location

[Isoform 1]: Widely expressed.

Background

IRF3 (interferon regulatory factor 3) is a member of the interferon regulatory transcription factor (IRF) family. The IRF3 gene is mapped on 19q13.33. IRF3 is found in an inactive cytoplasmic form that upon serine/threonine phosphorylation forms a complex with CREBBP. IRF3 plays an important role in the innate immune system's response to viral infection. Aggregated MAVS have been found to activate IRF3 dimerization. Although IRF3 increased transcriptional activity from an ISRE-containing promoter, expression of IRF3 as a Gal4 fusion protein did not activate expression of a chloramphenicol acetyltransferase (CAT) reporter gene containing repeats of the Gal4-binding sites. Translocation of IRF3 was accompanied by an increase in serine and threonine phosphorylation. The transcriptional activators CREBBP and EP300 coimmunoprecipitated with IRF3 only subsequent to viral infection, and the authors stated that these are also subunits of DRAF1.

Images

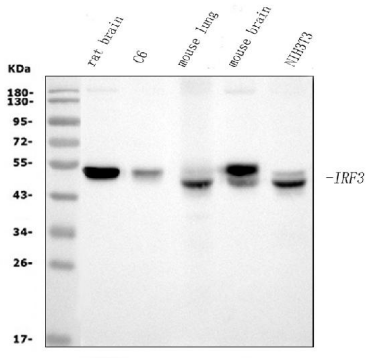


Figure 1. Western blot analysis of IRF3 using anti-IRF3 antibody (M00165-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: rat brain tissue lysates,
Lane 2: rat C6 whole cell lysates,
Lane 3: mouse lung tissue lysates,
Lane 4: mouse brain tissue lysates,
Lane 5: mouse NIH/3T3 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes.

Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-IRF3 antigen affinity purified monoclonal antibody (Catalog # M00165-3) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for IRF3 at approximately 50-55KD. The expected band size for IRF3 is at 50-55KD.

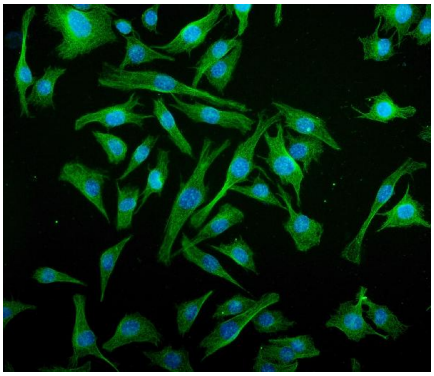


Figure 2. IF analysis of IRF3 using anti-IRF3 antibody (M00165-3).
IRF3 was detected in immunocytochemical section of RM-1 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-IRF3 Antibody (M00165-3) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

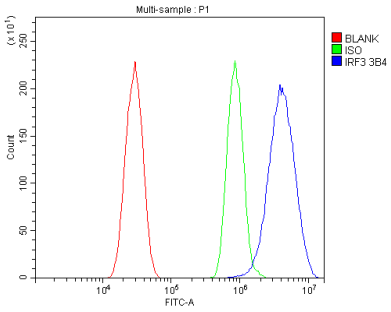


Figure 3. Flow Cytometry analysis of HEPA1-6 cells using anti-IRF3 antibody (M00165-3).
Overlay histogram showing HEPA1-6 cells stained with M00165-3 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-IRF3 Antibody (M00165-3, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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