abcam

ab315242

Anti-MDA5 antibody [EPR28350-21]

Recombinant | RabMAb | 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
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR28350-21
Purification technique	Affinity purification Protein A
Concentration	0.509 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Not recommended

Species	Rat, Human
Dilution info	-
Notes	-

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

Not recommended

Species	Rat, Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Not recommended

Species	Human, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/100
Notes	-

Not recommended

Species	Human, Rat
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information IFIH1

Function

Innate immune receptor which acts as a cytoplasmic sensor of viral nucleic acids and plays a major role in sensing viral infection and in the activation of a cascade of antiviral responses including the induction of type I interferons and pro-inflammatory cytokines (PubMed:28594402, PubMed:32169843, PubMed:33727702). Its ligands include mRNA lacking 2'-O-methylation at their 5' cap and long-dsRNA (>1 kb in length) (PubMed:22160685). Upon ligand binding it associates with mitochondria antiviral signaling protein (MAVS/IPS1) which activates the IKK-related kinases: TBK1 and IKBKE which phosphorylate interferon regulatory factors: IRF3 and IRF7 which in turn activate transcription of antiviral immunological genes, including interferons (IFNs); IFN-alpha and IFN-beta. Responsible for detecting the Picornaviridae family members such as encephalomyocarditis virus (EMCV), mengo encephalomyocarditis virus (ENMG), and rhinovirus (PubMed:28606988). Detects coronavirus SARS-CoV-2 (PubMed:33440148, PubMed:33514628). Can also detect other viruses such as dengue virus (DENV), west Nile virus (WNV), and reovirus. Also involved in antiviral signaling in response to viruses containing a dsDNA genome, such as vaccinia virus. Plays an important role in amplifying innate immune signaling through recognition of RNA metabolites that are produced during virus infection by ribonuclease L (RNase L). May play an important role in enhancing natural killer cell function and may be involved in growth inhibition and apoptosis in several tumor cell lines.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
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

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	MDA5 also known as melanoma differentiation-associated protein 5 or IFIH1 is a cytosolic sensor involved in the detection of viral RNA. It is a member of the RIG-I-like receptor family and possesses a molecular weight of approximately 116 kilodaltons. MDA5 protein is expressed across various tissues but shows higher expression in the immune system's cells including macrophages and dendritic cells. MDA5 recognizes long double-stranded RNA a common viral replication byproduct triggering immune responses.
Biological function summary	In cellular defense mechanisms MDA5 plays an important role in initiating antiviral responses. It operates as part of a signaling complex that includes MAVS (mitochondrial antiviral-signaling protein) leading to the production of type I

	interferons and pro-inflammatory cytokines. These responses promote an antiviral state in host cells aiding in the containment and clearance of viral infections. MDA5 antibodies can be useful tools for studying these immune processes.
Pathways	MDA5 interacts with antiviral signaling pathways such as the IFN signaling pathway and the RIG-I-like receptor signaling pathway. Upon recognizing viral RNA MDA-5 cooperates with proteins like RIG-I and MAVS to activate downstream signaling cascades that mediate immune responses. This collaboration enables efficient viral recognition and response limiting viral replication and spread.
Associated diseases and disorders	MDA5 has associations with autoimmune diseases like systemic lupus erythematosus and certain types of dermatomyositis. These conditions often involve autoantibodies against MDA5 indicating a possible role in pathogenesis. In these diseases altered function or regulation of related proteins including IFN-induced proteins may contribute to chronic inflammation and autoimmunity. Understanding MDA5's involvement in these contexts is essential for developing therapeutic strategies.

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

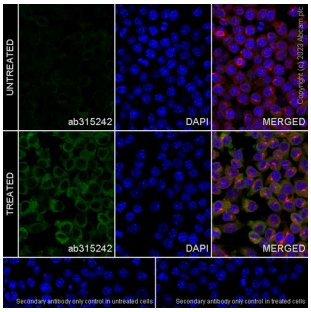
We do not recommend this combination. It is not covered by our product promise.

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.

6 product images



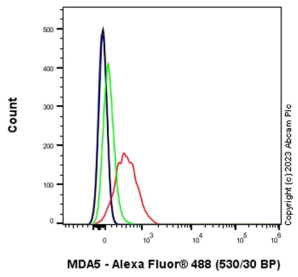
Immunocytochemistry/ Immunofluorescence - Anti-MDA5 antibody [EPR28350-21] (ab315242)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling MDA5 with ab315242 at 1/100 (5.09 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green).

Confocal image showing increased cytoplasmic staining in RAW 264.7 cells treated with IFNβ (2000 U/mL) for 24 hr. Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

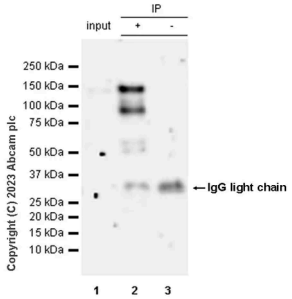
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.



Flow Cytometry (Intracellular) - Anti-MDA5 antibody [EPR28350-21] (ab315242)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 2000U/ml IFN beta for 24h (Red) / Untreated RAW 264.7 (Green) cells labelling MDA5 with ab315242 at 1/500 dilution (0.1 ug, Red and Green) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-MDA5 antibody [EPR28350-21] (ab315242)

MDA5 was immunoprecipitated from 0.35 mg Mouse spleen whole cell lysate with ab315242 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab315242 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: Mouse spleen whole cell lysate

Lane 2: ab315242 IP in Mouse spleen whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab315242 in mouse spleen whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-MDA5 antibody [EPR28350-21] (ab315242) at 1/30 dilution

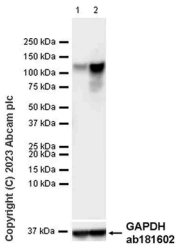
All lanes:
Mouse spleen whole cell lysate

Secondary

All lanes:

Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 180s



Western blot - Anti-MDA5 antibody [EPR28350-21] (ab315242)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-MDA5 antibody [EPR28350-21] (ab315242) at 1/1000 dilution

Lane 1:
Untreated RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

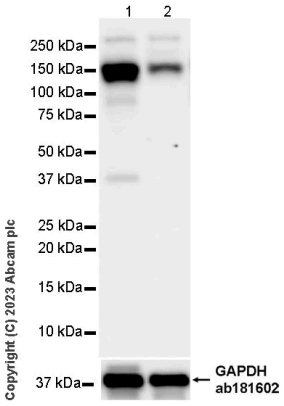
Lane 2:
RAW264.7 treated with 2000U/ml IFN beta for 24 hours whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 140 kDa, 36 kDa

Exposure time: 15s



Western blot - Anti-MDA5 antibody [EPR28350-21] (ab315242)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Low expression: testis (PMID: 12015121)

Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.

The identity of the higher MW band at approximately 250 kDa is unknown.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-MDA5 antibody [EPR28350-21] (ab315242) at 1/1000 dilution

Lane 1:
Mouse liver tissue lysate at 20 µg

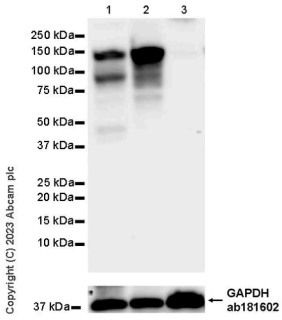
Lane 2:
Mouse testis tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 140 kDa, 90 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-MDA5 antibody [EPR28350-21] (ab315242)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Low expression: brain (PMID: 12015121)

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 17267501, 24390337).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-MDA5 antibody [EPR28350-21] (ab315242) at 1/1000 dilution

Lane 1:
Mouse spleen tissue lysate at 40 µg

Lane 2:
Mouse colon tissue lysate at 40 µg

Lane 3:
Mouse brain tissue lysate at 40 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 140 kDa, 90 kDa, 36 kDa

Exposure time: 180s

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