abcam

ab199247

Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR590(N)
Purification technique	Affinity purification Protein A
Dissociation constant	1.16 x 10^{-10} M
Concentration	1.004 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human

Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information MYD88

Function Adapter protein involved in the Toll-like receptor and IL-1 receptor signaling pathway in the innate immune response (PubMed:15361868, PubMed:18292575, PubMed:33718825, PubMed:37971847). Acts via IRAK1, IRAK2, IRF7 and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response (PubMed:15361868, PubMed:19506249, PubMed:24316379, PubMed:40638072). Increases IL-8 transcription (PubMed:9013863). Involved in IL-18-mediated signaling pathway. Activates IRF1 resulting in its rapid migration into the nucleus to mediate an efficient induction of IFN-beta, NOS2/INOS, and IL12A genes. Upon TLR8 activation by GU-rich single-stranded RNA (GU-rich RNA) derived

from viruses such as SARS-CoV-2, SARS-CoV and HIV-1, induces IL1B release through NLRP3 inflammasome activation (PubMed:33718825). MyD88-mediated signaling in intestinal epithelial cells is crucial for maintenance of gut homeostasis and controls the expression of the antimicrobial lectin REG3G in the small intestine (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab199247 is the carrier-free version of ab133739.

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.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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 conjugation kits 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.

Compatibility
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.

Supplementary info

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

Activity summary	MyD88 standing for myeloid differentiation primary response 88 is a cytoplasmic adaptor protein with a molecular weight of approximately 33 kDa. This protein is expressed widely in immune cells including monocytes macrophages and dendritic cells. MyD88 serves a major role in the signaling pathways for the innate immune system. It acts as a linker transmitting signals from toll-like receptors (TLRs) and interleukin-1 receptors (IL-1Rs) to downstream signaling molecules ultimately activating transcription factors.
Biological function summary	MyD88 plays a significant role in mediating immune responses by forming part of a complex that includes IRAK kinases and TRAF6. When TLRs or IL-1Rs activate MyD88 this adaptor protein recruits IRAK4 which then phosphorylates IRAK1 or IRAK2. This cascade promotes the activation of NF-κB and MAPK pathways leading to the production of inflammatory cytokines. The MyD88-dependent pathway is integral to innate immunity influencing how the body responds to pathogen infection and inflammation.
Pathways	MyD88 integrates into both the TLR signaling and IL-1R signaling pathways. Key related proteins in these pathways include interleukin-1 receptor-associated kinase (IRAK) and tumor necrosis factor receptor-associated factor 6 (TRAF6). MyD88 initiates the recruitment and activation of IRAK1 and IRAK4 following receptor engagement leading to subsequent activation of downstream signals. As part of these pathways MyD88 mediates cellular responses important for immune system signaling and inflammatory response regulation.
Associated diseases and disorders	MyD88 involvement is notable in the context of oncological and autoimmune diseases. Mutations or dysregulation of MyD88 are implicated in conditions like lymphoma and rheumatoid arthritis. In lymphoma the mutation usually results in constant activation of NF-κB leading to unchecked cell growth. As for rheumatoid arthritis an overactive immune response due to MyD88 can cause additional inflammation affecting joint tissues. In these conditions MyD88 interaction with IRAK4 is significant given their mutual role in immune and inflammatory pathways.

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.

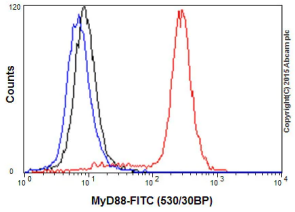
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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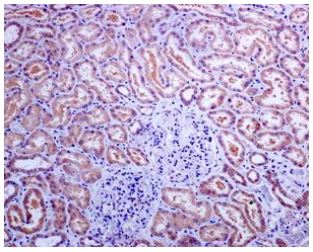
9 product images



Flow Cytometry (Intracellular) - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

MyD88 Flow Cytometry (Intracellular) staining using rabbit Anti-MyD88 antibody

Overlay histogram showing K562 cells fixed in 4% PFA and stained with purified ab133739 at a dilution of 1 in 350 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133739).

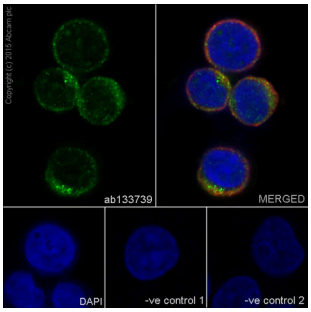


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

MyD88 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-MyD88 antibody

Immunohistochemistry analysis of Myd88 expression in formalin-fixed, paraffin-embedded Human kidney tissue, using unpurified ab133739 at 1/50 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133739).

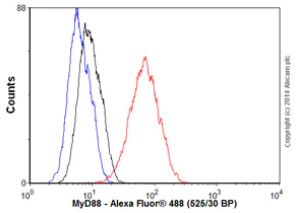
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

MyD88 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MyD88 antibody

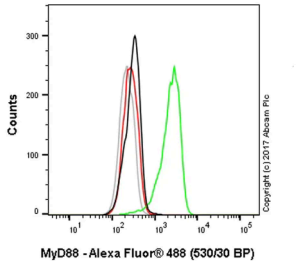
Immunofluorescence staining of Jurkat cells with purified ab133739 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab133739 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133739).



Flow Cytometry (Intracellular) - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

MyD88 Flow Cytometry (Intracellular) staining using rabbit Anti-MyD88 antibody

Overlay histogram showing MCF7 cells stained with unpurified ab133739 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab133739, 1/10000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in MCF7 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133739).



Flow Cytometry (Intracellular) - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

MyD88 Flow Cytometry (Intracellular) staining using rabbit Anti-MyD88 antibody

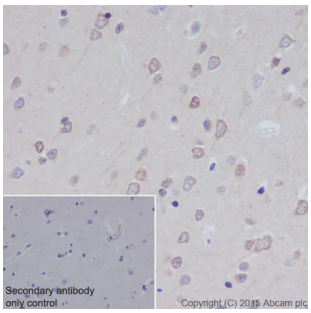
Overlay histogram showing HAP1 wildtype (green line) and HAP1-MyD88 knockout cells (red line) stained with ab133739. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab133739, 0.1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at 1/2000 dilution for 30 min at 22°C.

A rabbit IgG1 isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-MyD88 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30

bandpass filter.

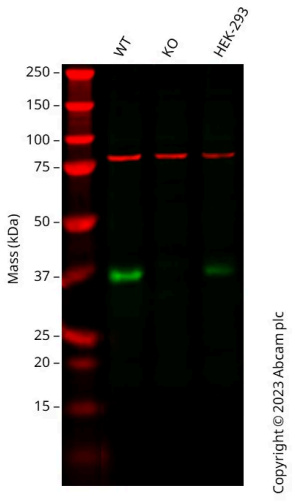
This antibody can also be used in HAP1 cells fixed with 4% formaldehyde (10 min) permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133739).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

MyD88 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-MyD88 antibody

Immunohistochemical staining of paraffin embedded human cerebral cortex with purified ab133739 at a working dilution of 1/500. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133739).



Western blot - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

MyD88 Western blot staining using rabbit Anti-MyD88 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133739). Western blot: Anti-MYD88 antibody [EPR590(N)] (ab133739) staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab133739 was shown to bind specifically to MYD88. A band was observed at 35 kDa in wild-type A549 cell lysates with no signal observed at this size in MYD88 knockout cell line ab286715 (knockout cell lysate ab290793). To generate this image, wild-type and MYD88 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% milk in TBS-0.1% Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
Lanes 1 - 3:
Western blot - Anti-MyD88 antibody [EPR590(N)] (ab133739) at 1/1000 dilution

Lanes 1 - 3:
Western blot - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

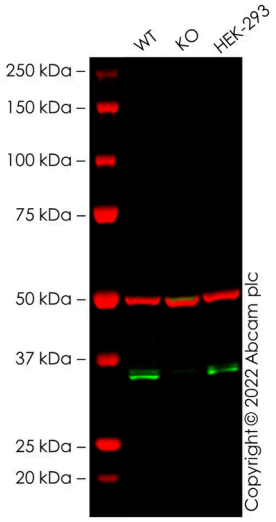
Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
MYD88 knockout A549 cell lysate at 20 µg

Lane 3:
HEK-293 cell lysate at 20 µg

Performed under reducing conditions.

Observed band size: 35 kDa



Western blot - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

MyD88 Western blot staining using rabbit Anti-MyD88 antibody

False colour image of Western blot: Anti-MyD88 antibody [EPR590(N)] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab133739 was shown to bind specifically to MyD88. A band was observed at 35 kDa in wild-type A549 cell lysates with no signal observed at this size in MYD88 knockout cell line ab286715 (knockout cell lysate ab290793). To generate this image, wild-type and MYD88 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133739).
All lanes:
Western blot - Anti-MyD88 antibody [EPR590(N)] (ab133739) at 1/1000 dilution

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

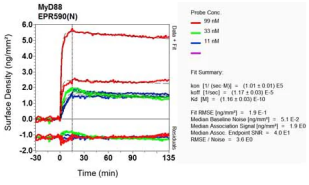
Lane 2:
MYD88 knockout A549 cell lysate at 20 µg

Lane 3:
HEK-293 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 33 kDa

Observed band size: 35 kDa



OI-RD Scanning - Anti-MyD88 antibody [EPR590(N)] - BSA and Azide free (ab199247)

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

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