abcam

Product datasheet

Anti-MyD88 antibody [EPR590(N)] ab133739

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 23 References 11 Images

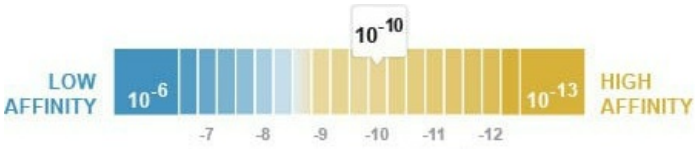
Overview

Product name	Anti-MyD88 antibody [EPR590(N)]
Description	Rabbit monoclonal [EPR590(N)] to MyD88
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Human kidney tissue. Jurkat, Molt-4, HepG2, K562, Raji cell lysates. Flow Cyt (intra): HAP1-WT cells
General notes	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**. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Stable for 12 months at -20°C.

Dissociation constant (K_D)	$K_D = 1.16 \times 10^{-10}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR590(N)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab133739 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

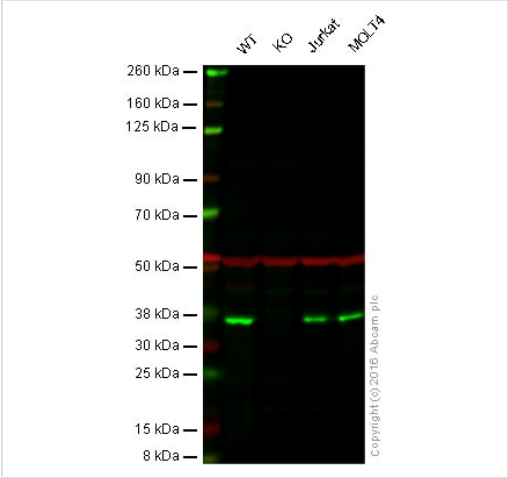
Application	Abreviews	Notes
Flow Cyt (Intra)		1/350. For unpurified use 1/500 - 1/10000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/500.
WB	★★★★★ (1)	1/1000 - 1/10000. Predicted molecular weight: 33 kDa.
IHC-P		1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **For unpurified, use 1/50 - 1/100.**

Target

Function	Adapter protein involved in the Toll-like receptor and IL-1 receptor signaling pathway in the innate immune response. Acts via IRAK1, IRAK2, IRF7 and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response. Increases IL-8 transcription. Involved in IL-18-mediated signaling pathway.
Tissue specificity	Ubiquitous.
Involvement in disease	Defects in MYD88 are the cause of MYD88 deficiency (MYD88D) [MIM:612260]; also known as recurrent pyogenic bacterial infections due to MYD88 deficiency. Patients suffer from autosomal recessive, life-threatening, often recurrent pyogenic bacterial infections, including invasive pneumococcal disease, and die between 1 and 11 months of age. Surviving patients are

	otherwise healthy, with normal resistance to other microbes, and their clinical status improved with age.
Sequence similarities	Contains 1 death domain. Contains 1 TIR domain.
Domain	The intermediate domain (ID) is required for the phosphorylation and activation of IRAK.
Cellular localization	Cytoplasm.

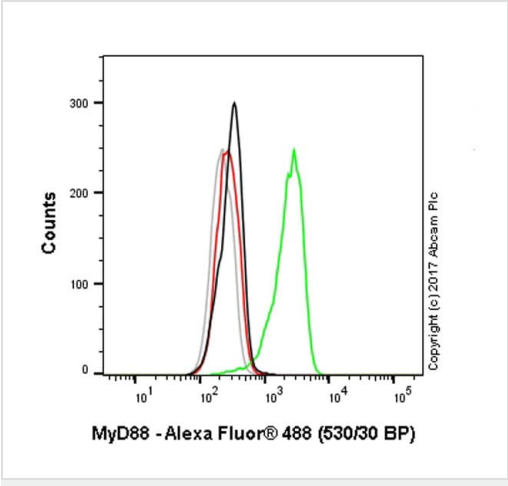
Images



Western blot - Anti-MyD88 antibody [EPR590(N)] (ab133739)

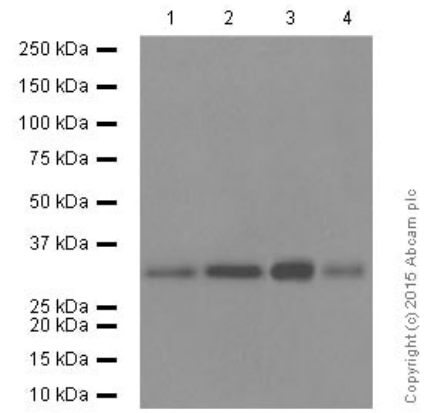
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: MyD88 knockout HAP1 cell lysate (20 µg)
Lane 3: Jurkat cell lysate (20 µg)
Lane 4: MOLT4 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab133739 observed at 37 kDa. Red - loading control, **ab7291**, observed at 52 kDa.

ab133739 was shown to specifically react with MyD88 when MyD88 knockout samples were used. Wild-type and MyD88 knockout samples were subjected to SDS-PAGE. ab133739 and **ab7291** (loading control to alpha tubulin) were 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 1/10000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-MyD88 antibody [EPR590(N)] (ab133739)

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.



Western blot - Anti-MyD88 antibody [EPR590(N)] (ab133739)

All lanes : Anti-MyD88 antibody [EPR590(N)] (ab133739) at 1/5000 dilution (purified)

Lane 1 : HepG2 cell lysate
Lane 2 : K562 cell lysate
Lane 3 : Raji cell lysate
Lane 4 : Jurkat cell lysate

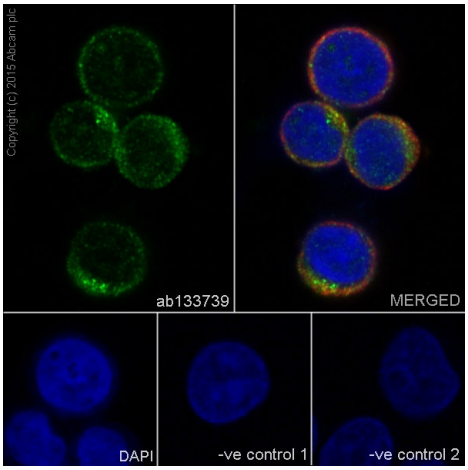
Lysates/proteins at 29 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 33 kDa
Observed band size: 33 kDa

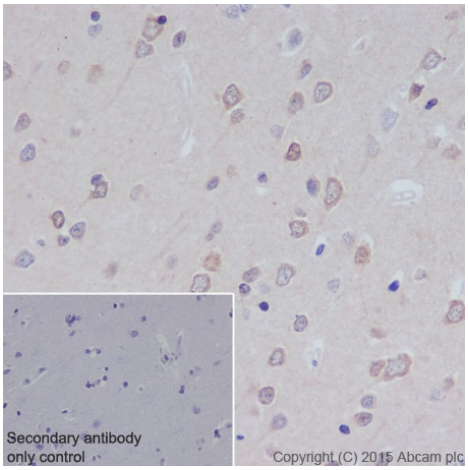
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



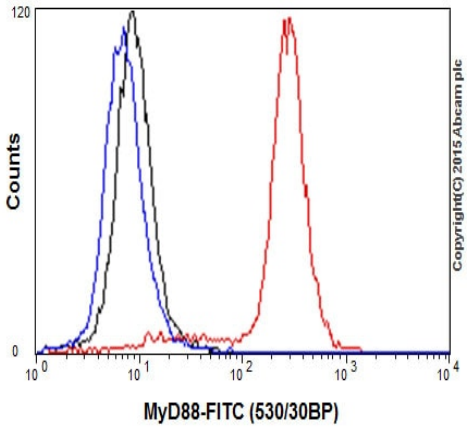
Immunocytochemistry/ Immunofluorescence - Anti-MyD88 antibody [EPR590(N)] (ab133739)

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.



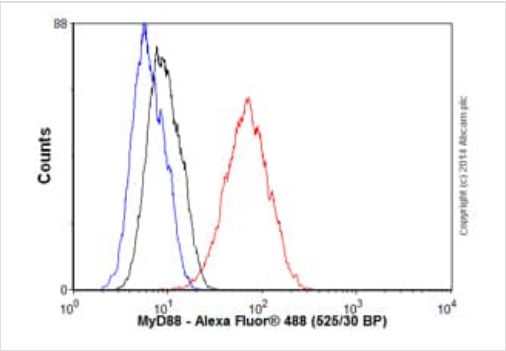
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MyD88 antibody [EPR590(N)] (ab133739)

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.



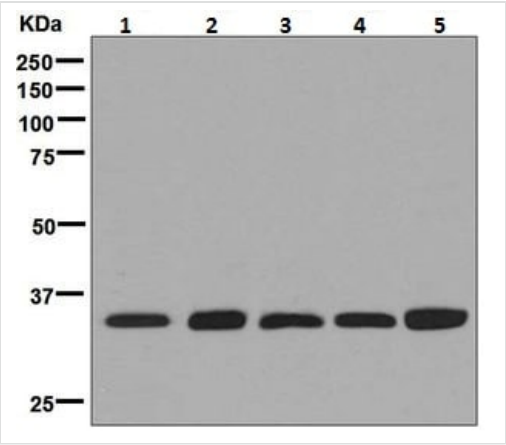
Flow Cytometry (Intracellular) - Anti-MyD88 antibody [EPR590(N)] (ab133739)

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).



Flow Cytometry (Intracellular) - Anti-MyD88 antibody [EPR590(N)] (ab133739)

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.



Western blot - Anti-MyD88 antibody [EPR590(N)] (ab133739)

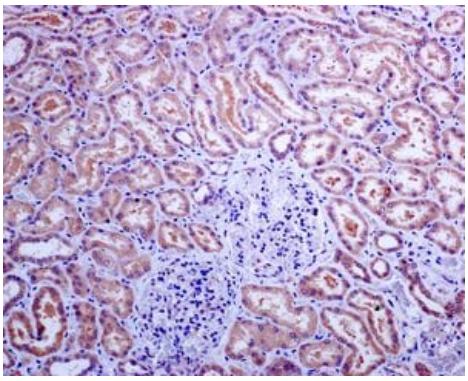
All lanes : Anti-MyD88 antibody [EPR590(N)] (ab133739) at 1/1000 dilution (unpurified)

Lane 1 : Jurkat cell lysate
Lane 2 : Molt-4 cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : K562 cell lysate
Lane 5 : Raji cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-rabbit at 1/2000 dilution

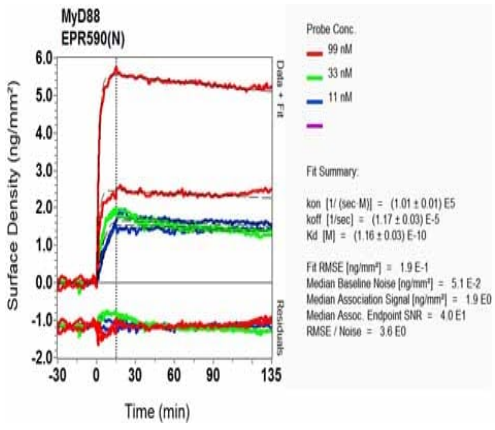
Predicted band size: 33 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MyD88 antibody [EPR590(N)] (ab133739)

Immunohistochemistry analysis of Myd88 expression in formalin-fixed, paraffin-embedded Human kidney tissue, using unpurified ab133739 at 1/50 dilution.

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



OI-RD Scanning - Anti-MyD88 antibody [EPR590(N)] (ab133739)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

Anti-MyD88 antibody [EPR590(N)] (ab133739)

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