abcam

Product datasheet

Anti-MARCO antibody [EPR22944-64] ab239369

Recombinant RabMAb

8 Images

Overview

Product name Anti-MARCO antibody [EPR22944-64]

Description Rabbit monoclonal [EPR22944-64] to MARCO

Host species Rabbit

Tested applications Suitable for: IP, IHC-Fr, IHC-P, ICC/IF, WB

Unsuitable for: Flow Cyt

Species reactivity Reacts with: Mouse

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

Positive control WB: J774A.1 treated with Lipopolysaccharides whole cell lysate. Mouse spleen lysate. Mouse

> spleen lysate treated with Peptide: N-glycosidase F (PNGase F). IHC-P: Mouse spleen and liver tissue. IHC-Fr: Mouse spleen tissue. ICC/IF: J774A.1 cells treated with Lipopolysaccharides. IP:

Mouse spleen lysate.

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**.

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. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR22944-64

Isotype IgG

Applications

The Abpromise guarantee O

Our <u>Abpromise guarantee</u> covers the use of ab239369 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
IP		1/30.
IHC-Fr		1/500.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.
WB		1/1000.

Application notes Is unsuitable for Flow Cyt.

Target

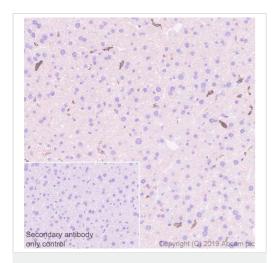
Function Pattern recognition receptor (PRR). Binds Gram-positive and Gram-negative bacteria.

Sequence similarities Contains 1 collagen-like domain.

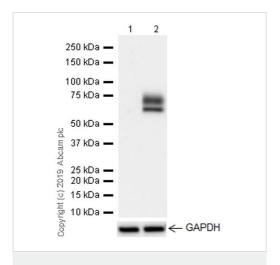
Contains 1 SRCR domain.

Cellular localization Membrane.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MARCO antibody
[EPR22944-64] (ab239369)



Western blot - Anti-MARCO antibody [EPR22944-64] (ab239369)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling MARCO with ab239369 at 1/500 dilution (1.11 µg/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on Kupffer cells of mouse liver is observed. The section was incubated with ab239369 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®]RX instrument. Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins.

All lanes : Anti-MARCO antibody [EPR22944-64] (ab239369) at 1/1000 dilution

Lane 1 : Untreated J774A.1 (mouse reticulum cell sarcoma monocyte macrophage), whole cell lysate

Lane 2: J774A.1 treated with 1ug/ml Lipopolysaccharides (LPS) for 24 hours, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

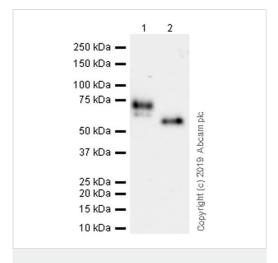
All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Observed band size: 55-75 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 seconds.

The expression of MARCO/Macrophage receptor with collagenous structure is induced by Lipopolysaccharides (LPS) treatment (PMID: 9916718).



Western blot - Anti-MARCO antibody [EPR22944-64] (ab239369)

All lanes : Anti-MARCO antibody [EPR22944-64] (ab239369) at 1/1000 dilution

Lane 1: Mouse spleen lysate

Lane 2 : Mouse spleen lysate treated with Peptide:N-glycosidase F (PNGase F)

Lysates/proteins at 20 µg per lane.

Secondary

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

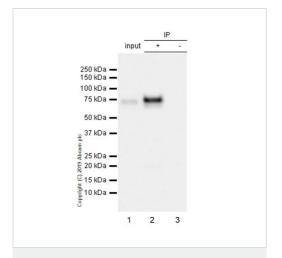
Observed band size: 53,75 kDa

Exposure time: 15 seconds

75 kDa (glycosylated from), 53 kDa (deglycosylated form).

Blocking and Dilution Buffer and concentration: 5% NFDM/TBST.

N-glycosylation is crucial for MARCO/Macrophage receptor with collagenous structure intercellular trafficking and surface targeting (PMID: 26892079).



Immunoprecipitation - Anti-MARCO antibody [EPR22944-64] (ab239369)

MARCO was immunoprecipitated from 0.35 mg mouse spleen lysate 10µg with ab239369 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab239369 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

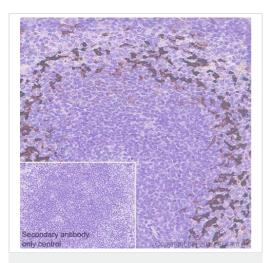
Lane 1: Mouse spleen lysate 10µg.

Lane 2: ab239369 IP in mouse spleen lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab239369 in mouse spleen lysate.

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

Exposure time: 8 seconds.

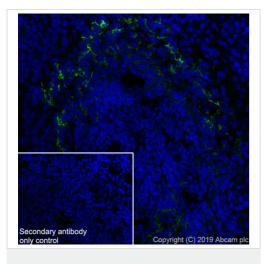


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MARCO antibody
[EPR22944-64] (ab239369)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling MARCO with ab239369 at 1/500 dilution (1.11 µg/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on macrophages of mouse spleen (PMID: 12874264, 15494482) is observed. The section was incubated with ab239369 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® instrument. Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

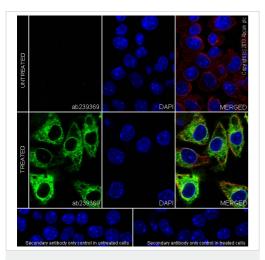
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins.



Immunohistochemistry (Frozen sections) - Anti-MARCO antibody [EPR22944-64] (ab239369)

4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen section of mouse spleen tissue labeling MARCO using ab239369 at 1/500 dilution, followed by **ab150077** AlexaFluor[®]488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (green). The nuclear counterstain is DAPI (blue). Positive staining on mouse splenic marginal zone (PMID: 24670793) is observed.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Immunocytochemistry/ Immunofluorescence - Anti-MARCO antibody [EPR22944-64] (ab239369)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized J744A.1 (Mouse reticulum cell sarcoma monocyte macrophage) cells labeling MARCO with ab239369 at 1/100 dilution, followed by ab150077 AlexaFluor 488 Goat anti-Rabbit secondary at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in J744A.1 cells treated with LPS (1 µg/ml) for 24h. The nuclear counterstain is DAPI (blue). Tubulin is detected with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor 594) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 dilution.



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