abcam

ab323568

Anti-CARD9 antibody [RM2079]

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	Multiclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM2079
Purification technique	Affinity purification Protein A
Specificity	Unsuitable for mouse IP and ICC
Concentration	0.491 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/50
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Not recommended

Species	Mouse
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
Notes	-

IP

Tested

Species	Human
Dilution info	1/30

Notes	-

Not recommended

Species	Mouse
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Mouse
Dilution info	-
Notes	-

Target data

See full target information CARD9

Function

Adapter protein that plays a key role in innate immune response against fungi by forming signaling complexes downstream of C-type lectin receptors (PubMed:26961233, PubMed:33558980). CARD9-mediated signals are essential for antifungal immunity against a subset of fungi from the phylum Ascomycota (PubMed:24231284, PubMed:25057046, PubMed:25702837, PubMed:26521038, PubMed:26679537, PubMed:26961233, PubMed:27777981, PubMed:29080677, PubMed:33558980). Transduces signals in myeloid cells downstream of C-type lectin receptors CLEC7A (dectin-1), CLEC6A (dectin-2) and CLEC4E (Mincle), which detect pathogen-associated molecular pattern metabolites (PAMPs), such as fungal carbohydrates, and trigger CARD9 activation (By similarity). Upon activation, CARD9 homooligomerizes to form a nucleating helical template that recruits BCL10 via CARD-CARD interaction, thereby promoting polymerization of BCL10 and subsequent recruitment of MALT1: this leads to activation of NF-kappa-B and MAP kinase p38 (MAPK11, MAPK12, MAPK13 and/or MAPK14) pathways which stimulate expression of genes encoding pro-inflammatory cytokines and chemokines (PubMed:11053425, PubMed:26488816, PubMed:26961233, PubMed:31296852, PubMed:33558980). CARD9 signaling in antigen-presenting cells links innate sensing of fungi to the activation of adaptive immunity and provides a cytokine milieu that induces the development and subsequent of interleukin 17-producing T helper (Th17) cells (PubMed:24231284). Also involved in activation of myeloid cells via classical ITAM-associated receptors and TLR: required for TLR-mediated activation of MAPK, while it is not required for TLR-induced activation of NF-kappa-B (By similarity). CARD9 can also be engaged independently of BCL10: forms a complex with RASGRF1 downstream of C-type lectin receptors, which recruits and activates HRAS, leading to ERK activation and the production of cytokines (By

similarity). Acts as an important regulator of the intestinal commensal fungi (mycobiota) component of the gut microbiota (PubMed:33548172). Plays an essential role in antifungal immunity against dissemination of gut fungi: acts by promoting induction of antifungal IgG antibodies response in CX3CR1(+) macrophages to confer protection against disseminated C.albicans or C.auris infection (PubMed:33548172). Also mediates immunity against other pathogens, such as certain bacteria, viruses and parasites; CARD9 signaling is however redundant with other innate immune responses (By similarity). In response to L.monocytogenes infection, required for the production of inflammatory cytokines activated by intracellular peptidoglycan: acts by connecting NOD2 recognition of peptidoglycan to downstream activation of MAP kinases (MAPK) without activating NF-kappa-B (By similarity).

Storage

Shipped at conditions	Blue Ice
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 recombinant multiclonals?
Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of heavy and light chains. They offer several advantages including:

- The sensitivity of polyclonal antibodies by recognising multiple epitopes
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

View our range of recombinant multiclonal antibodies.

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.

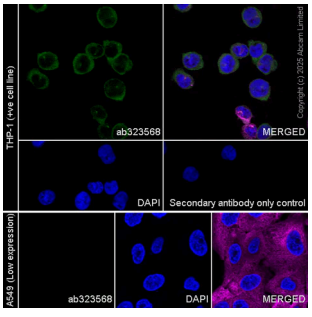
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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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7 product images



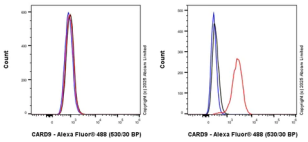
Immunocytochemistry/ Immunofluorescence - Anti-CARD9 antibody [RM2079] (ab323568)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized THP-1 (human monocytic leukemia monocyte) cells labelling CARD9 with ab323568 at 1/50 (9.82 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/ 1000 2ug/ml dilution (Green).

Confocal image showing cytoplasmic staining in THP-1 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue).
Low expression: A549 (PMID: 32164050).
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 2.5ug/ml dilution (Magenta). 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 2ug/ml dilution.

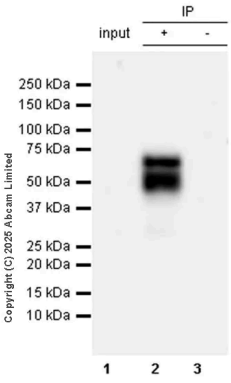


Flow Cytometry (Intracellular) - Anti-CARD9 antibody [RM2079] (ab323568)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized A549 (human lung carcinoma epithelial cell, Left) / THP-1 (human monocytic leukemia monocyte, Right) cells labelling CARD9 with ab323568 at 1/500 dilution (0.1 ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

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

Low expression: A549.



Immunoprecipitation - Anti-CARD9 antibody [RM2079] (ab323568)

CARD9 was immunoprecipitated from 0.35 mg THP-1 (human monocytic leukemia monocyte) whole cell lysate with ab323568 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab323568 at 1/1000 dilution.

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

All lanes:
Immunoprecipitation - Anti-CARD9 antibody [RM2079] (ab323568) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia monocyte) whole cell lysate at 10 µg

Lane 2:
ab323568 at 1/30 IP in THP-1 (human monocytic leukemia monocyte) whole cell lysate at 10 µg

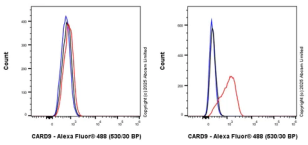
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab323568 in THP-1 whole cell lysate at 10 µg

Secondary

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

Observed band size: 43-62 kDa

Exposure time: 10s

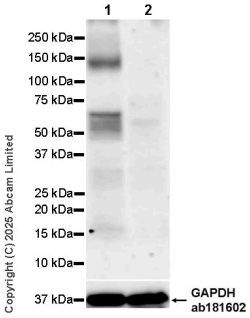


Flow Cytometry (Intracellular) - Anti-CARD9 antibody [RM2079] (ab323568)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized MEF (mouse embryo fibroblast, Left)/ RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage, Right) cells labelling CARD9 with ab323568 at 1/50 dilution (1 ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

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

Negative control: MEF.



Western blot - Anti-CARD9 antibody [RM2079] (ab323568)

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

Low expression: colon (PMID: 11053425).

The expression profile/ molecular weight observed is consistent with what has been described in the literature(PMID: 32164050), This antibody can recognize 3 isoforms of CARD9 and isoforms have different molecular weight.

The identity of the higher MW bands at about 150 kDa and the lower MW bands at about 15 kDa (in lane 1) are unknown

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

All lanes:
Western blot - Anti-CARD9 antibody [RM2079] (ab323568) at 1/1000 dilution

Lane 1:
Human tonsil tissue lysate at 40 µg

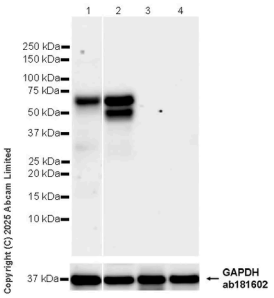
Lane 2:
Human colon tissues lysate at 40 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 43-62 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-CARD9 antibody [RM2079] (ab323568)

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

Low expression: A549 (PMID: 32164050), 293T (PMID: 33558980).

The expression profile/ molecular weight observed is consistent with what has been described in the literature(PMID: 32164050), This antibody can recognize 3 isoforms of CARD9 and isoforms have different molecular weight.

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

Exposure time: Lane 1: 81 seconds; Lanes 2-4: 26 seconds

All lanes:
Western blot - Anti-CARD9 antibody [RM2079] (ab323568) at 1/1000 dilution

Lane 1:
K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate at 20 µg

Lane 2:
THP-1 (human monocytic leukemia monocyte) whole cell lysate at 20 µg

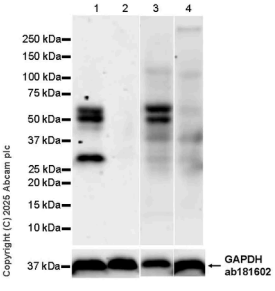
Lane 3:
A549 (human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 43-62 kDa, 36 kDa



Western blot - Anti-CARD9 antibody [RM2079] (ab323568)

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

Negative control: MEF.

Low expression: colon (PMID: 11053425).

The expression profile/ molecular weight observed is consistent with what has been described in the literature(PMID: 32164050), This antibody can recognize 3 isoforms of CARD9 and isoforms have different molecular weight.

The identity of the lower MW band at about 30 kDa (in lane 1) is unknown.

The identity of the higher MW bands at about 100 kDa and the lower MW bands at about 40 kDa (in lane 4) are unknown

This blot in lanes 3-4 was developed using a high-sensitivity ECL substrate, allowing for the detection of proteins in the mid-femtogram range.

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

Exposure time: Lanes 1-2: 180 seconds; Lanes 3-5: 75 seconds

All lanes:
Western blot - Anti-CARD9 antibody [RM2079] (ab323568) at 1/1000 dilution

Lane 1:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 40 µg

Lane 2:
MEF (mouse embryo fibroblast) whole cell lysate at 40 µg

Lane 3:
Mouse spleen tissue lysate at 40 µg

Lane 4:
Mouse colon lysate at 40 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 43-62 kDa, 36 kDa

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