abcam

ab318999

Anti-CD21+CD35 antibody [RM2062]

Recombinant | RabMAb | Advanced Validation | 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
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM2062
Purification technique	Affinity purification Protein A
Concentration	0.51 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Rat
Dilution info	-
Notes	-

mIHC

Tested

Species	Mouse
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Rat

Dilution info	-
Notes	-

Target data

See full target information Cr2

Function	Serves as a receptor for various ligands including complement component CD3d, HNRNPU OR IFNA1. When C3d is bound to antigens, attaches to C3d on B-cell surface and thereby facilitates the recognition and uptake of antigens by B-cells. This interaction enhances B-cell activation and subsequent immune responses. Forms a complex with several partners on the surface of B-cells including CD19, FCRL5 and CD81, to form the B-cell coreceptor complex that plays a crucial role in B-cell activation and signaling. Also induces specific intracellular signaling separately from the BCR and CD19 by activating the tyrosine kinase SRC, which then phosphorylates nucleolin/NCL and triggers AKT and GSK3 kinase activities in a SYK/CD19-independent manner. Acts as a ligand for CD23 (FcepsilonRII), a low-affinity receptor for IgE, which is expressed on B-cells and other immune cells, and thus participates in the regulation of IgE production.

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

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.

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.

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

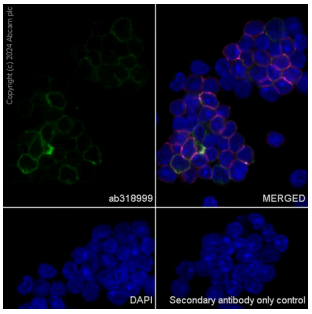
We do not recommend this combination. It is not covered by our product promise.

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.

8 product images



Immunocytochemistry/ Immunofluorescence - Anti-CD21+CD35 antibody [RM2062] (ab318999)

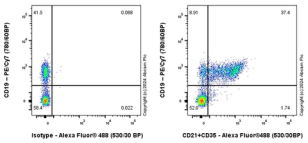
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Mouse splenocyte cells labelling CD21+CD35 with ab318999 at 1/50 (10.2 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/ml dilution (Green).

Confocal image showing membranous staining in mouse splenocytes (shown in green). The counterstain was CD45R/B220 which is observed in magenta. Nuclear DNA was labelled with DAPI

(shown in blue).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Anti-human/mouse CD45R/B220 rat monoclonal antibody (Alexa Fluor® 647) was used to counterstain tubulin at 1/100 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 2 ug/ml dilution.

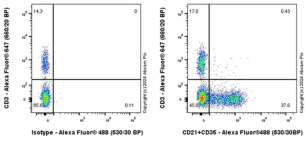


Flow Cytometry - Anti-CD21+CD35 antibody [RM2062] (ab318999)

Flow cytometric analysis of Mouse spleen cells labelling CD21+CD35 with ab318999 at 1/500 dilution (0.1 ug)/Right compared with a Rabbit monoclonal IgG (ab172730) / Left 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.

Mouse spleen cell are co-stained with CD19 conjugated PE/Cy7 (780/60BP).
Gated on viable cell.

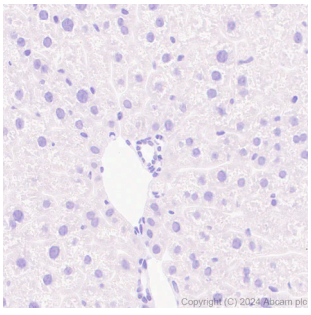


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Flow cytometric analysis of Mouse spleen cells labelling CD21+CD35 with ab318999 at 1/500 dilution (0.1 ug)/Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

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

Mouse spleen cell are co-stained with CD3 conjugated Alexa Fluor®647.
Gated on viable cell.


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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD21+CD35 antibody [RM2062] (ab318999)

CD21+CD35 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse liver using rabbit Anti-CD21+CD35 antibody

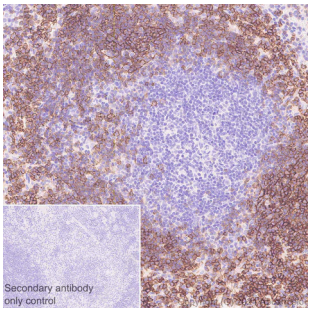
Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling CD21+CD35 with ab318999 at 1/2000 (0.255 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Negative control: no staining on mouse liver.
The section was incubated with ab318999 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD21+CD35 antibody [RM2062] (ab318999)

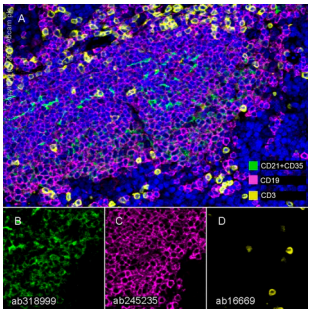
Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling CD21+CD35 with ab318999 at 1/2000 (0.255 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on mouse spleen.
The section was incubated with ab318999 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Multiplex immunohistochemistry - Anti-CD21+CD35 antibody [RM2062] (ab318999)

CD21+CD35 Multiplex immunohistochemistry staining of Mouse spleen using rabbit Anti-CD21+CD35 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse spleen tissue staining CD21+CD35 with ab318999 at a 1:2000 (0.255 ug/ml) dilution, ab245235 anti-CD19 used at 1:1000 (0.444 ug/ml) dilution and ab16669 anti-CD3 used at a 1:150 (0.06 ug/ml) dilution.

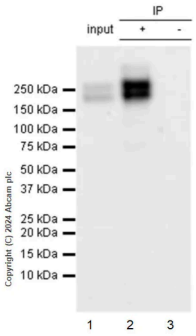
Panel A: merged staining of anti-CD21+CD35 (green; Opal™520), anti-CD19 (magenta; Opal™690) and anti-CD3 (yellow; Opal™570) on mouse spleen.
Panel B: anti-CD21+CD35 staining B lymphocytes in mouse spleen.
Panel C: anti-CD19 staining B lymphocytes in mouse spleen.
Panel D: anti-CD3 staining T lymphocytes in mouse spleen.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab318999, ab245235 and ab16669 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunoprecipitation - Anti-CD21+CD35 antibody [RM2062] (ab318999)

CD21+CD35 was immunoprecipitated from 0.35 mg Mouse spleen tissue lysate with ab318999 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab318999 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: Mouse spleen tissue lysate 10ug
Lane 2: ab318999 IP in Mouse spleen tissue lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab318999 in mouse spleen tissue lysate

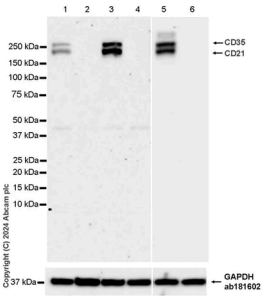
All lanes:
Immunoprecipitation - Anti-CD21+CD35 antibody [RM2062] (ab318999) at 1/30 dilution

All lanes:
Mouse spleen tissue lysate 10ug

Secondary

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

Exposure time: 15s



Western blot - Anti-CD21+CD35 antibody [RM2062] (ab318999)

CD21+CD35 Western blot staining using rabbit Anti-CD21+CD35 antibody

Negative control: EL4(PMID: 10453011)

Low expression: thymus, EL4.IL-2 (PMID: 2966205)

The identity of the lower MW band at approximately 37 kDa (in lane1- 4) is unknown.

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

Exposure time: Lane1-4: 180 seconds, Lane5-6: 26 seconds

All lanes:
Western blot - Anti-CD21+CD35 antibody [RM2062] (ab318999) at 1/1000 dilution

Lane 1:
WEHI-231 (mouse B cell lymphoma B lymphocyte) whole cell lysate at 20 µg

Lane 2:
EL4 (mouse lymphoma T lymphocyte) whole cell lysate at 20 µg

Lane 3:
A20 (mouse reticum sarcoma b lymphocyte) whole cell lysate at 20 µg

Lane 4:
EL4.IL-2 (mouse lymphoma T lymphocyte) whole cell lysate at 20 µg

Lane 5:
Mouse spleen tissue lysate at 20 µg

Lane 6:
Mouse thymus 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: 190 kDa, 250 kDa, 36 kDa

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