abcam

ab322534

Anti-CD69 antibody [RM1274]

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
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM1274
Purification technique	Affinity purification Protein A
Specificity	Unsuitable for mouse IHC, rat IHC, mouse WB and rat WB.
Concentration	0.515 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/500
Notes	

	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Species	Rat
Dilution info	1/500
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/500

Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information CD69

Function	Transmembrane protein expressed mainly on T-cells resident in mucosa that plays an essential role in immune cell homeostasis. Rapidly expressed on the surface of platelets, T-lymphocytes and NK cells upon activation by various stimuli, such as antigen recognition or cytokine signaling, stimulates different signaling pathways in different cell types (PubMed:24752896, PubMed:26296369, PubMed:35930205). Negatively regulates Th17 cell differentiation through its carbohydrate dependent interaction with galectin-1/LGALS1 present on immature dendritic cells (PubMed:24752896). Association of CD69 cytoplasmic tail with the JAK3/STAT5 signaling pathway regulates the transcription of RORgamma/RORC and, consequently, differentiation toward the Th17 lineage (By similarity). Also acts via the S100A8/S100A9 complex present on peripheral blood mononuclear cells to promote the conversion of naive CD4 T-cells into regulatory T-cells (PubMed:26296369). Acts as an oxidized low-density lipoprotein (oxLDL) receptor in CD4 T-lymphocytes and negatively regulates the inflammatory response by inducing the expression of PDCD1 through the activation of NFAT (PubMed:35930205). Participates in adipose tissue-derived mesenchymal stem cells (ASCs)-mediated protection against P.aeruginosa infection. Mechanistically, specifically recognizes P.aeruginosa to promote ERK1 activation, followed by granulocyte-macrophage colony-stimulating factor (GM-CSF) and other inflammatory cytokines secretion (PubMed:34841721). In eosinophils, induces IL-10 production through the ERK1/2 pathway (By similarity). Negatively regulates the chemotactic responses of effector lymphocytes and dendritic cells (DCs) to sphingosine 1 phosphate/S1P by acting as a S1PR1 receptor agonist and facilitating the internalization and degradation of the receptor (PubMed:37039481).

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

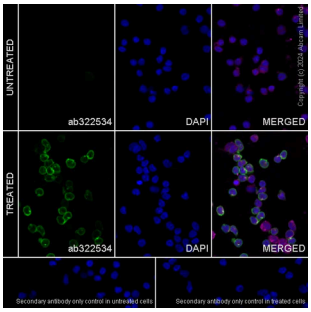
We do not recommend this combination. It is not covered by our product promise.

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.

10 product images



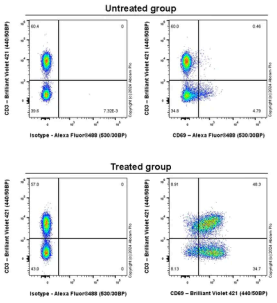
Immunocytochemistry/ Immunofluorescence - Anti-CD69 antibody [RM1274] (ab322534)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Human PBMC (human peripheral blood mononuclear cell) cells labelling CD69 with ab322534 at 1/500 (0.98 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 human PBMC (shown in green) treated with ionomycin (500 ng/mL) and phorbol-12-myristate-13-acetate (10 ng/mL) for 3 hr. The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). 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.

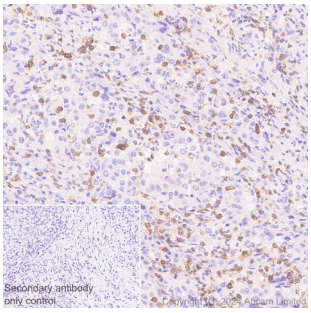


Flow Cytometry - Anti-CD69 antibody [RM1274] (ab322534)

Flow cytometric analysis of Human PBMC untreated with 300ng/mL ionomycin and 10ng/mL PMA for 3 hours(up left and up right) / Human PBMC treated with 300ng/mL ionomycin and 10ng/mL PMA for 3 hours(down left and down right) cells labelling CD69 with ab322534 at 1/500 dilution (0.1ug) / down right compared with a Rabbit monoclonal IgG (ab172730) / Left 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.

Human PBMC were stained with rabbit IgG or ab322534. Then stained with CD3 conjugated BV421 (450/50BP).
Gated on viable cell.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD69 antibody [RM1274] (ab322534)

Immunohistochemical analysis of paraffin-embedded Human gastric carcinoma tissue labeling CD69 with ab322534 at 1/500 (0.98 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

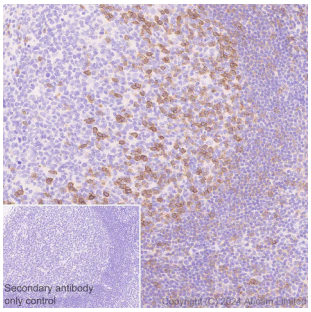
Positive staining on human gastric carcinoma. The section was incubated with ab322534 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

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

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD69 antibody [RM1274] (ab322534)

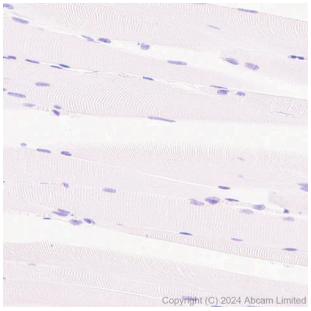
Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling CD69 with ab322534 at 1/500 (0.98 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human tonsil. The section was incubated with ab322534 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-CD69 antibody [RM1274] (ab322534)

CD69 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human skeletal muscle using rabbit Anti-CD69 antibody

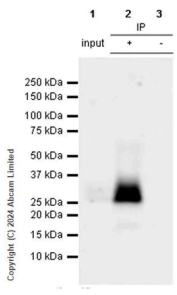
Immunohistochemical analysis of paraffin-embedded Human skeletal muscle tissue labeling CD69 with ab322534 at 1/500 (0.98 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Low expression: no staining on human skeletal muscle. The section was incubated with ab322534 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



Immunoprecipitation - Anti-CD69 antibody [RM1274] (ab322534)

CD69 was immunoprecipitated from 0.35 mg Daudi treated with 50ng/ml PMA for 24 hours, whole cell lysate with ab322534 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab322534 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: Daudi treated with 50ng/ml PMA for 24 hours, whole cell lysate
Lane 2: ab322534 IP in Daudi treated with 50ng/ml PMA for 24 hours, whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab322534 Daudi treated with 50ng/ml PMA for 24 hours, whole cell lysate

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

All lanes:

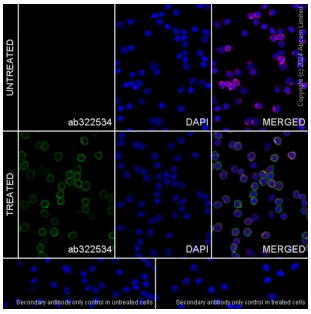
Immunoprecipitation - Anti-CD69 antibody [RM1274] (ab322534) at 1/30 dilution

All lanes:
Daudi treated with 50ng/ml PMA for 24 hours, whole cell lysate

Secondary

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

Exposure time: 10s



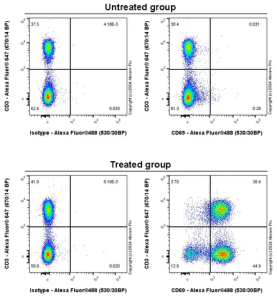
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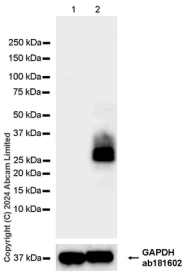


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Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Mouse PBMC were stained with rabbit IgG or ab322534. Then stained with CD3 conjugated Alexa Fluor®647 (670/14BP).
Gated on viable cell.



Western blot - Anti-CD69 antibody [RM1274] (ab322534)

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

The expression of CD69 is upregulated/downregulated in response to PMA treatment (PMID: 1617156).

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

All lanes:
Western blot - Anti-CD69 antibody [RM1274] (ab322534) at 1/1000 dilution

Lane 1:
Untreated Daudi (human Burkitts lymphoma lymphoblast) whole cell lysate at 20 µg

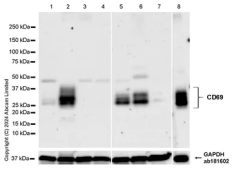
Lane 2:
Daudi treated with 50ng/ml PMA for 24 hours, whole cell lysate at 20 µg

Secondary

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

Observed band size: 28-32 kDa, 36 kDa

Exposure time: 15s



Western blot - Anti-CD69 antibody [RM1274] (ab322534)

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

Negative control: HeLa, 293T.

Low expression: K-562, skeletal muscle.

The identity of the higher MW band at approximately 50 kDa is unknown.

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

All lanes:
Western blot - Anti-CD69 antibody [RM1274] (ab322534) at 1/1000 dilution

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

Lane 2:
U-937 (human histiocytic lymphoma monocyte) whole cell lysate at 20 µg

Lane 3:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

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

Lane 5:
H9 (human lymphoma cutaneous T lymphocyte) whole cell lysate at 20 µg

Lane 6:
Human tonsil tissue lysate at 20 µg

Lane 7:
Human skeletal muscle tissue lysate at 20 µg

Lane 8:
Human thymus tissue lysate at 20 µg

Secondary

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

Observed band size: 28-32 kDa, 36 kDa

Exposure time: 125s

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