abcam

ab307082

Anti-CD69 antibody [EPR25398-81] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR25398-81
Purification technique	Affinity purification Protein A
Concentration	1.022 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Dot

Tested

Species	Recombinant full length protein - Human
Dilution info	-

Notes	-

Expected

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

IP

Expected

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

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

IHC-Fr

Tested

Species	Rat, Mouse

Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Rat, Mouse, Human, Recombinant full length protein - Human
Dilution info	-
Notes	-

WB

Not recommended

Species	Rat, Mouse, Human, Recombinant full length protein - Human
Dilution info	-
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 conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

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 the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CD69 also known as Activation-Induced C-type Lectin or Early T-cell Activation Antigen is a type II transmembrane protein with a mass of approximately 28-32 kDa. It is expressed mainly on activated leukocytes including T cells B cells and natural killer (NK) cells. Expression occurs rapidly after cell activation making CD69 a robust early marker used in flow cytometry analysis like with CD69 FITC or CD69 PE conjugates to study immune cell activation. The mouse version of CD69 serves as an important model for understanding its function in human biology.
Biological function summary	CD69 functions as a signaling molecule during the immune response influencing both cell proliferation and cytokine production. It is a member of the C-type lectin superfamily and operates as a homodimer on the cell surface. CD69 helps modulate the migration of T cells to inflammatory sites by affecting sphingosine-1-phosphate receptor 1 (S1P1) downregulation directing their tissue retention and primary response roles.
Pathways	The protein plays an important role in the regulation of the NF-kB and JAK-STAT signaling pathways. These are important for immune cell communication and response and CD69 interacts with proteins like the IL-2 receptor complex in these pathways. Additionally its cross-linking can lead to altered expression of cytokines and chemokines impacting overall immune function.
Associated diseases and disorders	Altered CD69 expression links to conditions like autoimmune diseases and chronic inflammation. Its involvement in rheumatoid arthritis shows how CD69 may regulate abnormal immune responses. Furthermore the modulation of CD69 expression can be associated with cancer progression highlighting connections to proteins like FN50 and FN61 which participate in tumor immunity and metastasis control.

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.

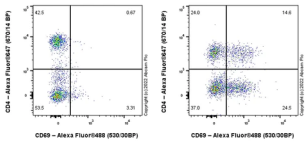
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

---

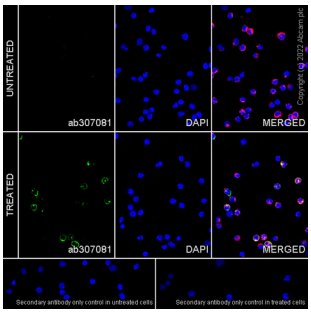
10 product images



Flow Cytometry - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

CD69 Flow Cytometry staining of Human peripheral blood mononuclear cell (PBMC) using rabbit Anti-CD69 antibody

This data was developed using ab307081, the same antibody clone in a different buffer formulation. Flow cytometric analysis of Human peripheral blood mononuclear cell (PBMC) treated with 500ng/ml ionomycin and 10ng/ml PMA for 3 hours (Right) / Untreated control (Left) cells lABelling CD69 with ab307081 at 1/500 dilution (0.1ug)/ Left and 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 1/2000 dilution was used as the secondary antibody. Cells were stained with Rabbit IgG or ab307081. Then stained with anti-CD4 conjugated to Alexa Fluor® 647.Gated on viable cells.

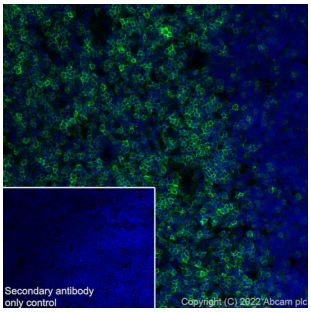


Immunocytochemistry/ Immunofluorescence - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

CD69 Immunocytochemistry/ Immunofluorescence staining of Human PBMC (human primary peripheral blood mononuclear cell) using rabbit Anti-CD69 antibody

This data was developed using ab307081, the same antibody clone in a different buffer formulation. Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Human PBMC (human primary peripheral blood mononuclear cell) cells labelling CD69 with ab307081 at 1/500 (1.036 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 weak cytoplasmic staining in subset of human PBMCs, the expression increased after

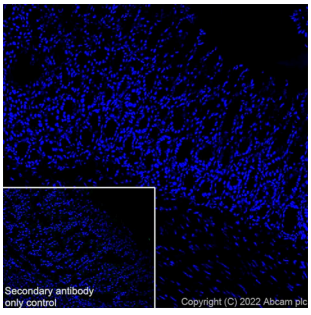
treatment with ionomycin (500 ng/ml) and PMA (10 ng/ml) for 3 hours. The image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5 ug/ml dilution (Red). 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.



Immunohistochemistry (Frozen sections) - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

CD69 Immunohistochemistry (Frozen sections) staining of Mouse thymus (fresh) using rabbit Anti-CD69 antibody

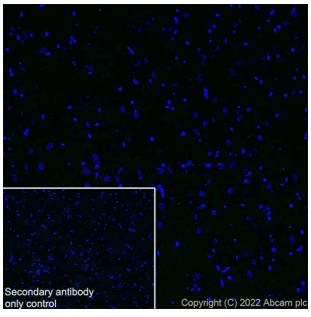
This data was developed using ab307081, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse thymus (fresh) tissue labeling CD69 with ab307081 at 1/100 (5.18 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution (Green). Confocal image showing positive staining on mouse thymus. The nuclear counterstain was DAPI (Blue). The section was incubated with ab307081 for 60 mins at room temperature. The section was then mounted using Fluoromount®. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). is observed. The nuclear counterstain was DAPI (Blue). Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution.



Immunohistochemistry (Frozen sections) - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

CD69 Immunohistochemistry (Frozen sections) staining of Mouse stomach (fresh) using rabbit Anti-CD69 antibody

This data was developed using ab307081, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse stomach (fresh) tissue labeling CD69 with ab307081 at 1/100 (5.18 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution (Green). Negative control: confocal image showing no staining on mouse stomach. The nuclear counterstain was DAPI (Blue). The section was incubated with ab307081 for 60 mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). is observed. The nuclear counterstain was DAPI (Blue). Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution.

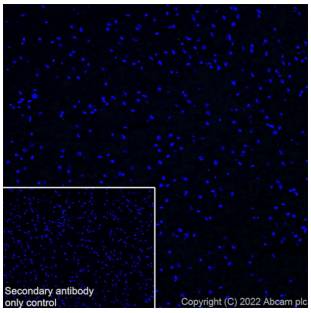


Immunohistochemistry (Frozen sections) - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

CD69 Immunohistochemistry (Frozen sections) staining of Rat cerebrum (fresh) using rabbit Anti-CD69 antibody

This data was developed using ab307081, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat cerebrum (fresh) tissue labeling CD69 with ab307081 at 1/100 (5.18 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution (Green). Negative control: confocal image showing no staining on rat cerebrum (PMID:

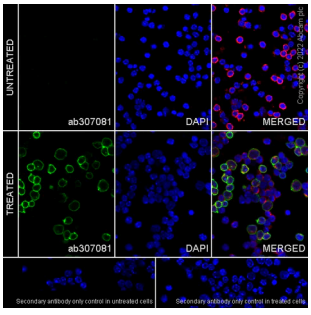
25970244). The nuclear counterstain was DAPI (Blue). The section was incubated with ab307081 for 60 mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). is observed. The nuclear counterstain was DAPI (Blue). Secondary antibody control: Secondary antibody is ab150081 Goat Anti-RABbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution.



Immunohistochemistry (Frozen sections) - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

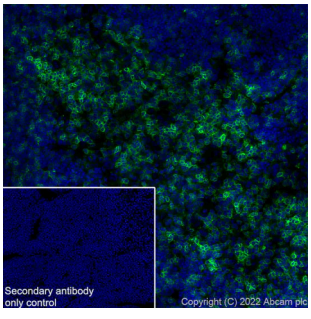
CD69 Immunohistochemistry (Frozen sections) staining of Mouse cerebrum (fresh) using rabbit Anti-CD69 antibody

This data was developed using ab307081, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse cerebrum (fresh) tissue labeling CD69 with ab307081 at 1/100 (5.18 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution (Green). Negative control: confocal image showing no staining on mouse cerebrum (PMID: 25970244). The nuclear counterstain was DAPI (Blue). The section was incubated with ab307081 for 60 mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). is observed. The nuclear counterstain was DAPI (Blue). Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution.



Immunocytochemistry/ Immunofluorescence - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

This data was developed using ab307081, the same antibody clone in a different buffer formulation. Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Mouse PBMC (mouse primary peripheral blood mononuclear cell) cells labelling CD69 with ab307081 at 1/500 (1.036 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 weak cytoplasmic staining in subset of mouse PBMCs, the expression increased after treatment with ionomycin (500 ng/ml) and PMA (10 ng/ml) for 24 hours. The image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor 594) was used to counterstain tubulin at 1/200 2.5 ug/ml dilution (Red). 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.

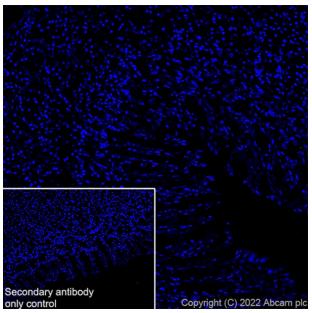


Immunohistochemistry (Frozen sections) - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

CD69 Immunohistochemistry (Frozen sections) staining of Rat thymus (fresh) using rabbit Anti-CD69 antibody

This data was developed using ab307081, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat thymus (fresh) tissue labeling CD69 with ab307081 at 1/100 (5.18 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution (Green). Confocal image showing positive staining on rat thymus. The nuclear counterstain was DAPI (Blue). The section was incubated with ab307081 for 60 mins at room temperature. The section was then mounted using Fluoromount®. The immunostaining was

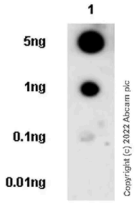
performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). is observed. The nuclear counterstain was DAPI (Blue). Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution.



Immunohistochemistry (Frozen sections) - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

CD69 Immunohistochemistry (Frozen sections) staining of Rat stomach (fresh) using rabbit Anti-CD69 antibody

This data was developed using ab307081, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat stomach (fresh) tissue labeling CD69 with ab307081 at 1/100 (5.18 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution (Green). Negative control: confocal image showing no staining on rat stomach. The nuclear counterstain was DAPI (Blue). The section was incubated with ab307081 for 60 mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). is observed. The nuclear counterstain was DAPI (Blue). Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution.



Dot Blot - Anti-CD69 antibody [EPR25398-81] - BSA and Azide free (ab307082)

This data was developed using ab307081, the same antibody clone in a different buffer formulation. Dot blot analysis of CD69 using ab307081 at 1:1000 (0.518 ug/ml) followed by a Goat Anti-RABbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1:100,000 dilution. Exposure time: 180 seconds Blocking and diluting buffer and concentration: 5% NFDM/TBST

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