abcam

ab317458

Anti-Granzyme B antibody [RM1165]

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	RM1165
Purification technique	Affinity purification Protein A
Concentration	0.519 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse

Dilution info	1/1000
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/1000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Species	Mouse
Dilution info	1/30
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Expected

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

Not recommended

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/50

Notes	-

Species	Human
Dilution info	1/50
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IHC-Fr

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information GZMB

Function Abundant protease in the cytosolic granules of cytotoxic T-cells and NK-cells which activates caspase-independent pyroptosis when delivered into the target cell through the immunological synapse (PubMed:1985927, PubMed:3262682, PubMed:3263427). It cleaves after Asp (PubMed:1985927, PubMed:8258716). Once delivered into the target cell, acts by catalyzing cleavage of gasdermin-E (GSDME), releasing the pore-forming moiety of GSDME, thereby triggering pyroptosis and target cell death (PubMed:31953257, PubMed:32188940). Seems to be linked to an activation cascade of caspases (aspartate-specific cysteine proteases) responsible for apoptosis execution. Cleaves caspase-3, -9 and -10 (CASP3, CASP9 and CASP10, respectively) to give rise to active enzymes mediating apoptosis (PubMed:9852092). Cleaves and activates CASP7 in response to bacterial infection, promoting plasma membrane repair (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

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.

Supplementary info

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

Activity summary	Granzyme B also known as GZMB GB11 or granzyme B protein is a serine protease with a molecular mass of approximately 32 kDa. It is expressed mainly in cytotoxic T lymphocytes and natural killer (NK) cells. This enzyme plays a mechanical role in inducing apoptosis in target cells serving as an effector protein in the immune system's defense against virally infected cells or transformed cancer cells. The activity of granzyme B relies on its ability to cleave after aspartate residues in substrate proteins leading to the activation of apoptotic pathways.
Biological function summary	Granzyme B participates prominently in the immune response by activating caspases particularly caspase-3 which promotes the breakdown of cellular components necessary for apoptosis. Granzyme B does not function in isolation but acts in concert with other immune system factors such as perforin to effectively induce cell death. Perforin creates pores in the target cell membrane allowing granzyme B to enter and instigate the apoptosis sequence. The enzyme

	also contributes to the processing of cytokines which enhances the immune response further.
Pathways	Studies have determined that granzyme B is critical in the apoptosis pathway particularly in the granule exocytosis pathway. It closely interacts with proteins such as perforin and other granzymes to mediate apoptosis in target cells. Granzyme B also plays a role in the inflammatory response and can influence pathways associated with cytotoxic T cell signaling. Its pathway interactions ensure effective elimination of damaged or infected cells maintaining tissue homeostasis.
Associated diseases and disorders	Granzyme B has associations with autoimmune diseases and cancer. Abnormally high levels of granzyme B can contribute to tissue damage and inflammation in autoimmune conditions like rheumatoid arthritis. In the context of cancer granzyme B aids in tumor surveillance and destruction when functioning correctly but impaired granzyme B activity can lead to evasion of immune detection by cancerous cells. Perforin also plays a role in these conditions closely working with granzyme B to either protect against or drive disease progression.

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

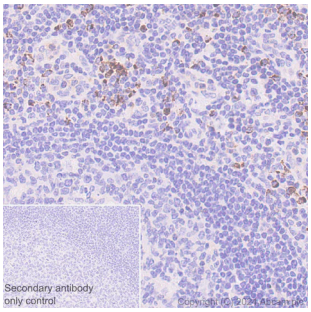
We do not recommend this combination. It is not covered by our product promise.

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.

11 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Granzyme B antibody [RM1165] (ab317458)

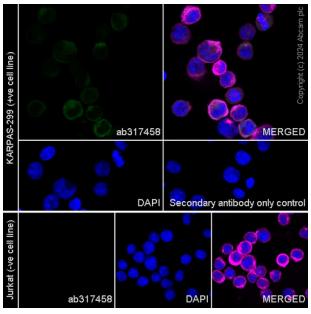
Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling Granzyme B with ab317458 at 1/1000 (0.51 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human tonsil. The section was incubated with ab317458 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



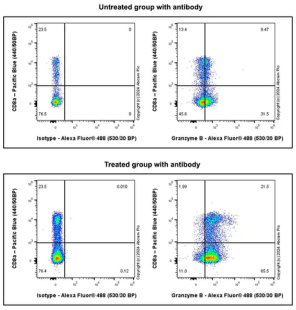
Immunocytochemistry/ Immunofluorescence - Anti-Granzyme B antibody [RM1165] (ab317458)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized KARPAS-299 (human T cell lymphoma cell) cells labelling Granzyme B with ab317458 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 cytoplasmic staining in KARPAS-299 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labeled with DAPI (shown in blue).
Negative control: Jurkat (PMID:18437383).
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 2.5 ug/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.

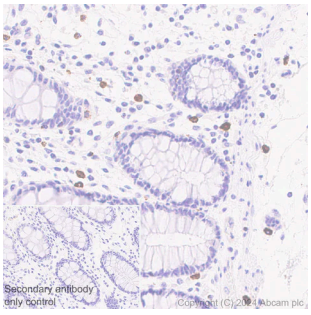


Flow Cytometry (Intracellular) - Anti-Granzyme B antibody [RM1165] (ab317458)

Flow cytometric analysis of 2% paraformaldehyde fixed 0.1% Tween-20 permeabilized Human PBMC(human peripheral blood mononuclear cell) treated with 10ug/ml CD3 for 72hours and 10ug/ml CD28 for 72 hours(Bottom left and Bottom right) / Untreated Human PBMC(Top left and Top right) cells labelling Granzyme B with ab317458 at 1/50 dilution (1 ug)/Top right and Bottom right (Red) 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.

human PBMC are co-stained with CD8a conjugated Pacific Blue (440/50BP).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Granzyme B antibody [RM1165] (ab317458)

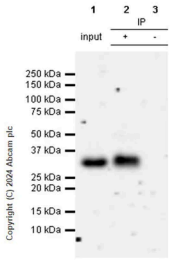
Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Granzyme B with ab317458 at 1/1000 (0.51 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on immune cells of human colon. The section was incubated with ab317458 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-Granzyme B antibody [RM1165] (ab317458)

Granzyme B was immunoprecipitated from 0.35 mg KARPAS-299 (human T cell lymphoma cell) whole cell lysate with ab317458 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab317458 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: KARPAS-299 (human T cell lymphoma cell) whole cell lysate
Lane 2: ab317458 IP in KARPAS-299 (human T cell lymphoma cell) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab317458 in KARPAS-299 whole cell lysate

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

All lanes:
Immunoprecipitation - Anti-Granzyme B antibody [RM1165] (ab317458) at 1/30 dilution

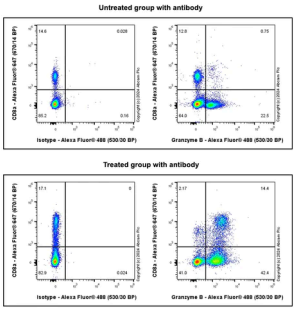
Lane 1:
KARPAS-299 (human T cell lymphoma cell) whole cell lysate

Lane 2:
ab317458 IP in KARPAS-299 (human T cell lymphoma cell) whole cell lysate

Secondary

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

Exposure time: 180s

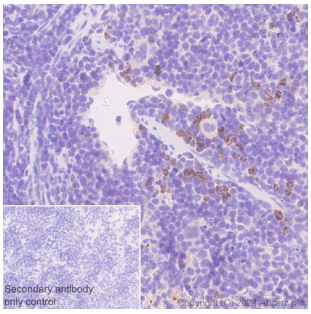


Flow Cytometry (Intracellular) - Anti-Granzyme B antibody [RM1165] (ab317458)

Flow cytometric analysis of 2% paraformaldehyde fixed 0.1% Tween-20 permeabilized Mouse PBMC(mouse peripheral blood mononuclear cell) treated with 2.5ug/ml Concanavalin A for 72h (Bottom left and Bottom right) / Untreated mouse PBMC(Top left and Top right) cells labelling Granzyme B with ab317458 at 1/50 dilution (1 ug)/Top right and Bottom right (Red) 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 PBMC are co-stained with CD8a conjugated Pacific Blue (440/50BP).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Granzyme B antibody [RM1165] (ab317458)

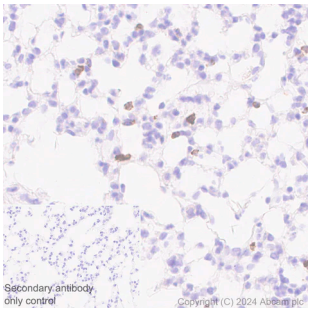
Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling Granzyme B with ab317458 at 1/2000 (0.255 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on mouse spleen. The section was incubated with ab317458 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-Granzyme B antibody [RM1165] (ab317458)

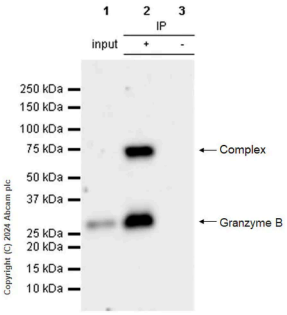
Immunohistochemical analysis of paraffin-embedded Mouse lung tissue labeling Granzyme B with ab317458 at 1/2000 (0.255 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on immune cells of mouse lung. The section was incubated with ab317458 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-Granzyme B antibody [RM1165] (ab317458)

Granzyme B was immunoprecipitated from 0.35 mg Mouse spleen cell treated with 2.5 ug/ml Concanavalin A for 72h whole cell lysate with ab317458 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab317458 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: Mouse spleen cell treated with 2.5 ug/ml Concanavalin A for 72h whole cell lysate
Lane 2: ab317458 IP in Mouse spleen cell treated with 2.5 ug/ml Concanavalin A for 72h whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab317458 in mouse spleen cell treated with 2.5 ug/ml Concanavalin A for 72h whole cell lysate

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

All lanes:
Immunoprecipitation - Anti-Granzyme B antibody [RM1165] (ab317458) at 1/30 dilution

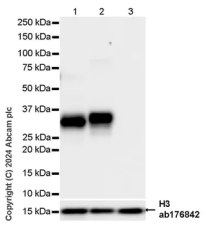
Lane 1:
Mouse spleen cell treated with 2.5 ug/ml Concanavalin A for 72h whole cell lysate

Lane 2:
ab317458 IP in Mouse spleen cell treated with 2.5 ug/ml Concanavalin A for 72h whole cell lysate

Secondary

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

Exposure time: 41s



Western blot - Anti-Granzyme B antibody [RM1165] (ab317458)

Negative control: Jurkat (PMID:18437383).

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

All lanes:
Western blot - Anti-Granzyme B antibody [RM1165] (ab317458) at 1/1000 dilution

Lane 1:
KARPAS-299 (human T cell lymphoma cell) whole cell lysate at 20 µg

Lane 2:
SR (human pleural effusion lymphoblast) whole cell lysate at 20 µg

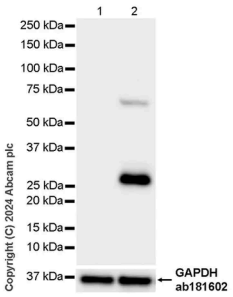
Lane 3:
Jurkat (human T cell leukemia T lymphocyte from peripheral blood) whole cell lysate at 20 µg

Secondary

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

Observed band size: 28 kDa, 15 kDa

Exposure time: 15s



Western blot - Anti-Granzyme B antibody [RM1165] (ab317458)

The molecular weight observed is consistent with what has been described in the literature (PMID: 8663264 and 17116752).

Granzyme B forms complex with its protease inhibitor (PMID:9774654).

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

All lanes:
Western blot - Anti-Granzyme B antibody [RM1165] (ab317458) at 1/1000 dilution

Lane 1:
Untreated mouse splenocyte lysate at 20 µg

Lane 2:
Mouse splenocytes treated with 2.5µg/ml concanavalin for 72 hours 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 kDa, 36 kDa

Exposure time: 15s

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