abcam

Product datasheet

Anti-Granzyme B antibody [EPR20129-217] ab208586

Recombinant RabMAb

3 References 10 Images

Overview

Product name Anti-Granzyme B antibody [EPR20129-217]

Description Rabbit monoclonal [EPR20129-217] to Granzyme B

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, mIHC

Unsuitable for: Flow Cyt

Reacts with: Human Species reactivity

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human Granzyme B recombinant protein; IHC-P: Human colon and cervix cancer tissues.

mIHC: Human breast cancer tissue.

General notes 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 production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb patents**.

Properties

Form Liquid

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long Storage instructions

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR20129-217

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab208586 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/10000. Detects a band of approximately 28 kDa (predicted molecular weight: 28 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
mIHC		Use at an assay dependent concentration.

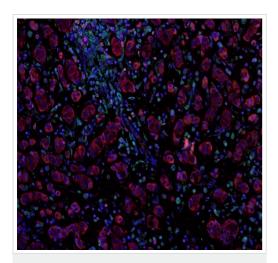
Application notes

Is unsuitable for Flow Cyt.

Target

Function	This enzyme is necessary for target cell lysis in cell-mediated immune responses. It cleaves after			
	Asp. Seems to be linked to an activation cascade of caspases (aspartate-specific cysteine			
	proteases) responsible for apoptosis execution. Cleaves caspase-3, -7, -9 and 10 to give rise to			
	active enzymes mediating apoptosis.			
Sequence similarities	Belongs to the peptidase S1 family. Granzyme subfamily.			
	Contains 1 peptidase S1 domain.			
Cellular localization	Cytoplasmic granule. Cytoplasmic granules of cytolytic T-lymphocytes and natural killer cells.			

Images



Multiplex immunohistochemistry - Anti-Granzyme B antibody [EPR20129-217] (ab208586)

This image is courtesy of ImmunoAtlas.

Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of Anti-PD-L1 (ab251611; cyan; Opal[™] 520), Anti-Granzyme B (ab219803; yellow; Opal[™] 540), Anti-PD1 (ab251613; magenta; Opal[™] 570), Anti-pan Cytokeratin (ab264485; red; Opal[™] 620), Anti-EpCAM (ab225894; red; Opal[™] 620), Anti-CD8 alpha (ab251596; green; Opal[™] 650) and Anti-FOXP3 (ab96048; orange; Opal[™] 690). EpCAM and pancytokeratin share the same dye and color. Dyes are pseudocolored for better contrast of the markers.

The immunostaining was performed on a Leica Biosystems BOND® MAX instrument with an Opal™ 6-Plex Detection Kit (NEL821001KT, Akoya Biosciences®).

The section was incubated in six rounds of staining; sequentially for

ab251611 (1/750 dilution), ab219803 (1/250 dilution), ab251613 (1/750 dilution), ab264485 (0.5 μg/ml), ab225894 (1/1250 dilution), ab251596 (1/1500 dilution) and ab96048 (10 μg/ml); each using a separate fluorescent tyramide signal amplification system. EDTA based antigen retrieval (Leica Biosystems BOND® Epitope Retrieval Solution 2, pH 9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity. DAPI (dark blue) was used as a nuclear counter stain.

Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra 3 Imaging System (Akoya Biosciences®).

This data is courtesy of ImmunoAtlas and it can be found **here**.

1 2
250 kDa —
150 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
37 kDa —
31 kDa —
31 kDa —
31 kDa —
31 kDa —
4 His-tag

Western blot - Anti-Granzyme B antibody [EPR20129-217] (ab208586) **All lanes :** Anti-Granzyme B antibody [EPR20129-217] (ab208586) at 1/10000 dilution

Lane 1 : Human Granzyme B recombinant protein

Lane 2 : Human Granzyme H recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary

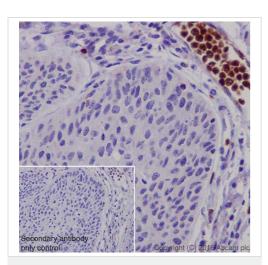
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 28 kDa
Observed band size: 26 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

Human Granzyme B and human Granzyme H recombinant protein contain aa21-247 and aa21-246 with a His-tag. These two recombinant proteins were made in house.

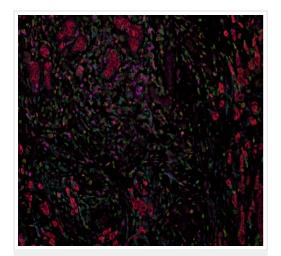


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Granzyme B antibody
[EPR20129-217] (ab208586)

Immunohistochemical analysis of paraffin-embedded human cervix cancer tissue labeling Granzyme B with ab208586 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on neutrophils and stroma cells of human cervix cancer is observed [PMID: 14512315]. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Multiplex immunohistochemistry - Anti-Granzyme B antibody [EPR20129-217] (ab208586)

This image is courtesy of ImmunoAtlas.

Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of Anti-PD-L1 (ab251611; cyan; Opal™ 520), Anti-Granzyme B (ab219803; yellow; Opal™ 540), Anti-PD1 (ab251613; magenta; Opal™ 570), Anti-pan Cytokeratin (ab264485; red; Opal™ 620), Anti-EpCAM (ab225894; red; Opal™ 620), Anti-CD8 alpha (ab251596; green; Opal™ 650) and Anti-FOXP3 (ab96048; orange; Opal™ 690). EpCAM and pancytokeratin share the same dye and color. Dyes are pseudocolored for better contrast of the markers.

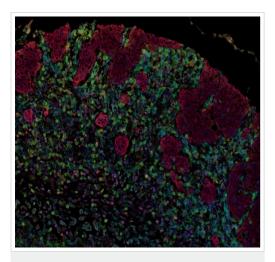
The immunostaining was performed on a Leica Biosystems ${\sf BOND}^{\circledR} \, {\sf MAX} \, \text{instrument with an Opal}^{\intercal} \, {\sf 6-Plex} \, {\sf Detection} \, {\sf Kit} \, \\ ({\sf NEL821001KT}, \, {\sf Akoya} \, {\sf Biosciences}^{\circledR}).$

The section was incubated in six rounds of staining; sequentially for ab251611 (1/750 dilution), ab251613 (1/250 dilution), ab264485 (0.5 μg/ml), ab225894 (1/1250 dilution), ab251596 (1/1500 dilution) and ab96048 (10 μg/ml); each using a separate fluorescent tyramide signal amplification system. EDTA based antigen retrieval (Leica Biosystems BOND® Epitope Retrieval Solution 2, pH 9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from

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Multiplex immunohistochemistry - Anti-Granzyme B antibody [EPR20129-217] (ab208586)

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Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

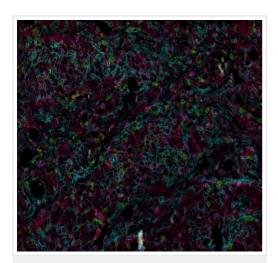
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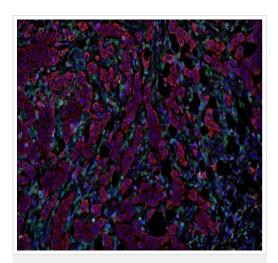
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Multiplex immunohistochemistry - Anti-Granzyme B antibody [EPR20129-217] (ab208586)

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Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

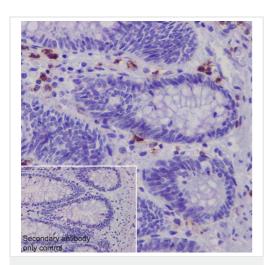
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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Granzyme B antibody
[EPR20129-217] (ab208586)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Granzyme B with ab208586 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on some stromal cells of human colon is observed. Counter stained with Hematoxylin.

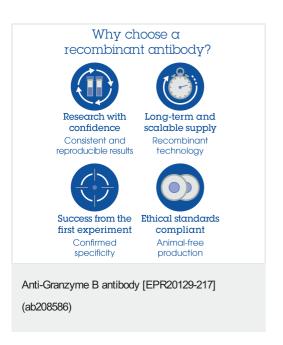
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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

Normal fissue samples				Malignant tissue samples			
Human cardiac muscle	x	Human placenta	×	Clear cell carcinoma of human kidney	x [mmune cells √]	Human glioma	x
Human cerebrum	x	Human skeletal muscle	×	Human bladder cancer	x	Human hepatocellular carcinoma	× (immune cells √
Human colon	x (immune cells √)	Human skin	×	Human breast carcinoma	≭ [immune cells √]	Human lung carcinoma	x
Human endometrium	x	Human spleen	✓	Human cervical carcinoma	x [immune cells √]	Human ovarian carcinoma	× (immune cells ✓
Human kidney	x	Human stomach	×	Human colon carcinoma	x [immune cells √]	Human pancreatic carcinoma	x (immune cells √
Human liver	x	Human festis	x	Human endometrial carcinoma	▼ [immune cells √]	Human prostatic hyperplasia	x (immune cells √
Human lung	x	Human thyroid	×	Human gastric adenocarcinoma	▼ [immune cells √]	Human thyroid carcinoma	x [immune cells √
Human mammary gland	x	Human tonsil	✓				

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Granzyme B antibody
[EPR20129-217] (ab208586)

Tissue Microarrays stained for "Anti-Granzyme B antibody [EPR20129-217]" using "ab208586" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab208586 for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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