abcam

Product datasheet

Anti-CD8 alpha antibody [EPR21769] - BSA and Azide free ab230156



10 Images

Overview

Product name Anti-CD8 alpha antibody [EPR21769] - BSA and Azide free

Description Rabbit monoclonal [EPR21769] to CD8 alpha - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IHC-P, WB, IHC-Fr, Flow Cyt, IP

Species reactivity Reacts with: Mouse

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: Mouse spleen tissue.

General notes ab230156 is the carrier-free version of ab217344.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased 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.

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.

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

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR21769

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab230156 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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Background staining was observed in mouse testis samples.
WB		Use at an assay dependent concentration. Detects a band of approximately 34-38 kDa (predicted molecular weight: 27 kDa).
IHC-Fr		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Target

modifications

Function Identifies cytotoxic/suppressor T-cells that interact with MHC class I bearing targets. CD8 is

thought to play a role in the process of T-cell mediated killing. CD8 alpha chains binds to class I

MHC molecules alpha-3 domains.

Involvement in disease Defects in CD8A are a cause of familial CD8 deficiency (CD8 deficiency) [MIM:608957]. Familial

CD8 deficiency is a novel autosomal recessive immunologic defect characterized by absence of

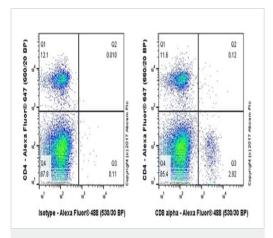
CD8+ cells, leading to recurrent bacterial infections.

Sequence similarities Contains 1 lg-like V-type (immunoglobulin-like) domain.

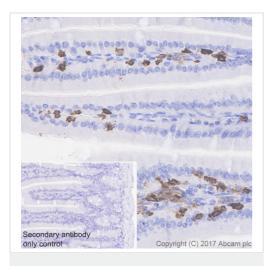
Post-translational All of the five most carboxyl-terminal cysteines form inter-chain disulfide bonds in dimers and

higher multimers, while the four N-terminal cysteines do not.

Cellular localization Secreted and Cell membrane.



Flow Cytometry - Anti-CD8 alpha antibody [EPR21769] - BSA and Azide free (ab230156)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD8 alpha antibody
[EPR21769] - BSA and Azide free (ab230156)

Flow cytometric analysis of mouse primary splenocytes labeling CD8 alpha with <u>ab217344</u> at 1/500 dilution (right panel) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) (left panel). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (<u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

Cells were surface stained with CD4-Alexa Fluor[®] 647, then stained with rabbit IgG (Left) / **ab217344** (Right) separately. CD4 and CD8 alpha are mutually exclusive expressed in mouse spleen. Gated on total viable cells.

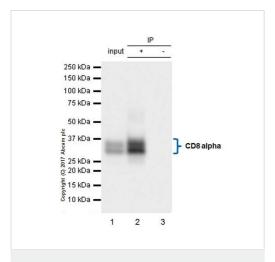
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217344).

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling CD8 alpha with <u>ab217344</u> at 1/2000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Positive staining on stromal cells of mouse colon. Counter stained with Hematoxylin.

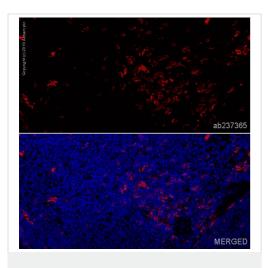
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217344).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-CD8 alpha antibody [EPR21769] - BSA and Azide free (ab230156)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD8 alpha antibody

[EPR21769] - BSA and Azide free (ab230156)

CD8 alpha was immunoprecipitated from 0.35 mg of mouse thymus lysate with <u>ab217344</u> at 1/30 dilution. Western blot was performed from the immunoprecipitate using <u>ab217344</u> at 1/2000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: Mouse thymus lysate 10 µg (Input).

Lane 2: ab217344 IP in mouse thymus lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of $\underline{ab214344}$ in mouse thymus lysate.

Exposure time: 5 seconds.

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

The two bands are different isoforms that are consistent with the literature (PMID 3085089).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217344).

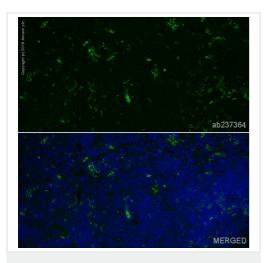
Clone EPR21769 (ab230156) has been successfully conjugated by Abcam. This image was generated using Anti-CD8 alpha antibody [EPR21769] (Alexa Fluor® 647). Please refer to ab237365 for protocol details.

IHC image of CD8 alpha staining in a section of formalin-fixed paraffin-embedded normal mouse spleen.

The section was pre-treated using heat mediated antigen retrieval with Tris/EDTA buffer (pH9, epitope retrieval solution 2) for 20mins, performed on a Leica BOND[™]. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with ab237365 at 1/100 dilution (shown in red). Nuclear DNA was labeled with DAPI (shown in blue). The section was then mounted using Fluoromount[®].

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

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD8 alpha antibody
[EPR21769] - BSA and Azide free (ab230156)

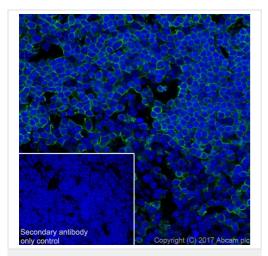
Clone EPR21769 (ab230156) has been successfully conjugated by Abcam. This image was generated using Anti-CD8 alpha antibody [EPR21769] (Alexa Fluor® 488). Please refer to ab237364 for protocol details.

IHC image of CD8 alpha staining in a section of formalin-fixed paraffin-embedded normal mouse spleen.

The section was pre-treated using heat mediated antigen retrieval with Tris/EDTA buffer (pH9, epitope retrieval solution 2) for 20mins, performed on a Leica BOND [™]. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with ab237364 at 1/100 dilution (shown in green). Nuclear DNA was labeled with DAPI (shown in blue). The section was then mounted using Fluoromount[®].

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

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.



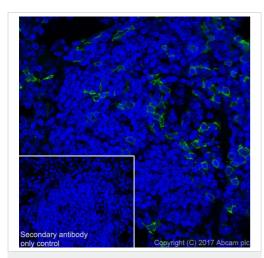
Immunohistochemistry (Frozen sections) - Anti-CD8 alpha antibody [EPR21769] - BSA and Azide free (ab230156)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse thymus tissue labeling CD8 alpha with ab217344 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Positive membrane staining on mouse thymus tissue section (PMID: 25616911).

The nuclear counter stain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217344).



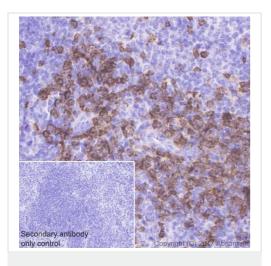
Immunohistochemistry (Frozen sections) - Anti-CD8 alpha antibody [EPR21769] - BSA and Azide free (ab230156)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse spleen tissue labeling CD8 alpha with ab217344 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Positive membrane staining on mouse spleen (PMID: 25616911).

The nuclear counter stain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217344).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD8 alpha antibody

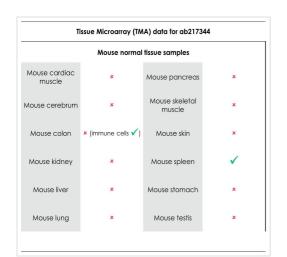
[EPR21769] - BSA and Azide free (ab230156)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling CD8 alpha with <u>ab217344</u> at 1/2000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Positive staining on the white pulp of mouse spleen (PMID: 23482450; PMID: 25826597). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217344).

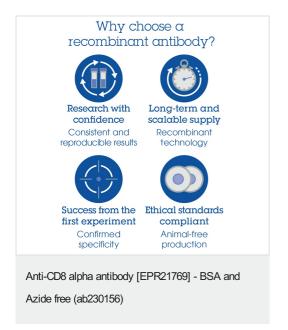
Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD8 alpha antibody

[EPR21769] - BSA and Azide free (ab230156)

Tissue Microarrays stained for "Anti-CD8 alpha antibody [EPR21769]" using "ab217344" 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 ab93684 (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab217344 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



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