abcam

ab237709

Anti-CD8 alpha antibody [CAL66]

Anti-CD8 alpha antibody [CAL66] (ab237709) is a rabbit monoclonal antibody detecting CD8 alpha in **Flow Cytometry (Intra), IP, IHC-P, ICC/IF, mIHC**. Suitable for **Human, Rat**.

- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency
- Over 40 publications

Recombinant | RabMAb | Advanced Validation | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.05% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	CAL66
Purification technique	Affinity purification Protein A
Concentration	0.482 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purity >99%

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Rat, Cat, Dog, Cow
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Rat, Cat, Dog, Cow
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Not recommended

Species	Rat, Human, Cat, Dog, Cow
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Expected

Species	Rat, Cat, Dog, Cow
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Rat
Dilution info	1 µg/mL
Notes	Use at 1 µg/ml for rat Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Human
Dilution info	0.25 µg/mL
Notes	Use at 1 µg/ml for rat Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Cat
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Dog
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Cow
Dilution info	1/100

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

mIHC

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Rat, Cat, Dog, Cow
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information CD8A

Function

Integral membrane glycoprotein that plays an essential role in the immune response and serves multiple functions in responses against both external and internal offenses. In T-cells, functions primarily as a coreceptor for MHC class I molecule:peptide complex. The antigens presented by class I peptides are derived from cytosolic proteins while class II derived from extracellular proteins. Interacts simultaneously with the T-cell receptor (TCR) and the MHC class I proteins presented by antigen presenting cells (APCs). In turn, recruits the Src kinase LCK to the vicinity of the TCR-CD3 complex. LCK then initiates different intracellular signaling pathways by phosphorylating various substrates ultimately leading to lymphokine production, motility, adhesion and activation of cytotoxic T-lymphocytes (CTLs). This mechanism enables CTLs to recognize and eliminate infected cells and tumor cells. In NK-cells, the presence of CD8A homodimers at the cell surface provides a survival mechanism allowing conjugation and lysis of multiple target cells. CD8A homodimer molecules also promote the survival and differentiation of activated lymphocytes into memory CD8 T-cells.

Storage

Shipped at conditions	Conditional Ambient
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

Product Specifications
Anti-CD8 alpha antibody [CAL66] (ab237709) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, IP, mIHC in human, rat samples.
Anti-CD8 alpha antibody [CAL66] (ab237709) specifically detects CD8 alpha (UniProt ID: P01732; Molecular weight: 24kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Quality and Validation
Abcam's high quality manufacturing and validation processes ensure Anti-CD8 alpha antibody [CAL66] (ab237709) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.
Anti-CD8 alpha antibody [CAL66] (ab237709) has been cited over 43 times in peer reviewed journals and is trusted by the scientific community.

Related Products
Conjugation-ready, carrier free format available for antibody clone CAL66 - ab251596.
Antibody clone CAL66 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 647, Alexa Fluor® 488, Alexa Fluor® 555, Alexa Fluor® 594 (ab305048, ab305220, ab305367, ab305370).

Target Information
CD8 alpha is a transmembrane glycoprotein that functions as a co-receptor for the T-cell receptor (TCR), mainly found on cytotoxic T cells. It plays a crucial role in T cell signaling and cytotoxic activity by binding to MHC class I molecules, aiding the immune response against infected or malignant cells. CD8 alpha is associated with several diseases, particularly those involving immune system dysfunction

Supplementary info

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

Activity summary	CD8 alpha also known as CD8A or CD8 protein is a glycoprotein subunit expressed on the surface of cytotoxic T lymphocytes. It has a mass of approximately 32 kDa. Found on the surface cell membrane CD8 alpha functions primarily in the immune response specifically in the recognition of antigens bound to major histocompatibility complex (MHC) class I molecules. Often scientists use CD8 antibodies for detection and CD8 IHC or immunohistochemistry for localization studies.

Biological function summary	The CD8 alpha protein plays a critical role in T-cell mediated immune responses. It forms a heterodimer with the CD8 beta chain creating the CD8 alpha-beta complex that strengthens T-cell interaction with antigen-presenting cells. CD8 alpha also helps in signaling processes that activate T cells equipping them to destroy infected or malignant cells. Researchers often study CD8 alpha peptides to understand its interactions better.
Pathways	CD8 alpha is integral to the T-cell receptor signaling pathway and the cytotoxic T lymphocyte (CTL) pathway. The T-cell receptor complex which includes the CD8 molecule transmits signals that are important for T-cell activation and function. CD8 interacts with key proteins such as the T-cell receptor (TCR) and MHC class I molecules facilitating targeted responses against pathogens. These pathways highlight CD8 alpha's role in adaptive immunity.
Associated diseases and disorders	CD8 alpha is most prominently associated with viral infections and cancer. Conditions like HIV and some forms of leukemia show altered CD8 function highlighting the protein's role in immune surveillance. In HIV infection for instance CD8 T cells reduce in number impairing the immune response. CD8 alpha's connection to the immune system places it alongside other immune proteins such as CD4 and MHC molecules in the context of immune dysfunction.

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

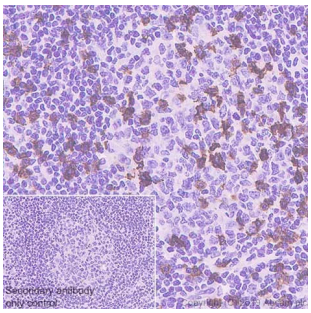
We do not recommend this combination. It is not covered by our product promise.

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.

15 product images

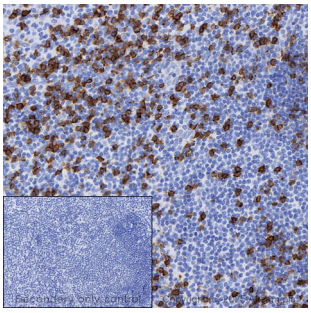


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD8 alpha antibody [CAL66] (ab237709)

CD8 alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD8 alpha antibody

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD8 alpha with ab237709 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Positive staining on the human tonsil is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).



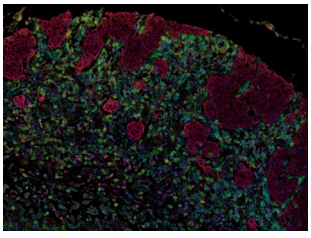
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD8 alpha antibody [CAL66] (ab237709)

CD8 alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human Tonsil tissue using rabbit Anti-CD8 alpha antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling CD8 alpha with ab237709 at a concentration of 0.1µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

ab237709 anti-CD8 alpha antibody [CAL66] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



This image is courtesy of ImmunoAtlas.

Multiplex immunohistochemistry - Anti-CD8 alpha antibody [CAL66] (ab237709)

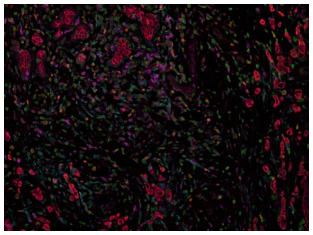
CD8 alpha Multiplex immunohistochemistry staining using rabbit Anti-CD8 alpha antibody

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 pan-cytokeratin share the same dye and color. Dyes are pseudo-colored 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.



This image is courtesy of ImmunoAtlas.

Multiplex immunohistochemistry - Anti-CD8 alpha antibody [CAL66] (ab237709)

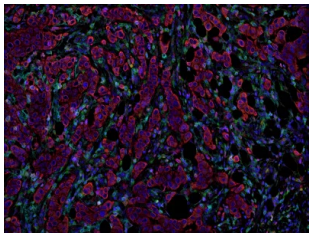
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Multiplex immunohistochemistry - Anti-CD8 alpha antibody [CAL66] (ab237709)

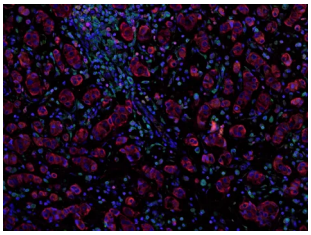
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Multiplex immunohistochemistry - Anti-CD8 alpha antibody [CAL66] (ab237709)

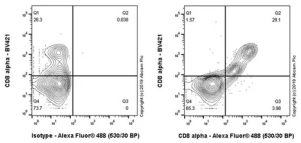
CD8 alpha Multiplex immunohistochemistry staining using rabbit Anti-CD8 alpha antibody

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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 pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

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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®).

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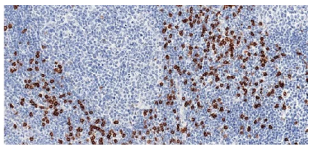


Flow Cytometry (Intracellular) - Anti-CD8 alpha antibody [CAL66] (ab237709)

CD8 alpha Flow Cytometry (Intracellular) staining of Human PBMC (peripheral blood mononuclear cells) using rabbit Anti-CD8 alpha antibody

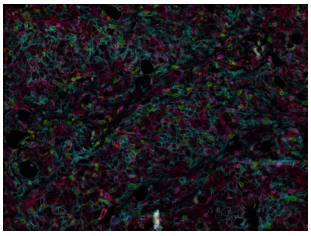
Flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween 20 permeabilized human PBMC (peripheral blood mononuclear cells) labeling CD8 alpha with ab237709 at 1/500 dilution (Right) compared with a Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Left).
Goat Anti-Rabbit IgG Fc (Alexa Fluor® 488) preadsorbed (ab150097), at 1/5000 dilution was used as the secondary antibody.

Cells were surface stained with anti-CD8 alpha conjugated to BV421.The fixed with 2% PFA for 10min followed by intracellularly stained with rabbit IgG (Left) or ab237709 (Right). They recognize same populations.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD8 alpha antibody [CAL66] (ab237709)

Formalin-fixed, paraffin-embedded human tonsil tissue stained for CD8 alpha using ab237709 at 0.25 µg/ml in immunohistochemical analysis.



This image is courtesy of ImmunoAtlas.

Multiplex immunohistochemistry - Anti-CD8 alpha antibody [CAL66] (ab237709)

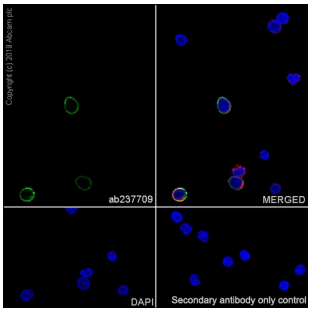
CD8 alpha Multiplex immunohistochemistry staining using rabbit Anti-CD8 alpha antibody

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 pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

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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®).

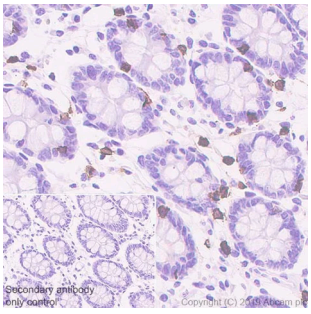
This data is courtesy of ImmunoAtlas and it can be found here.



Immunocytochemistry/ Immunofluorescence - Anti-CD8 alpha antibody [CAL66] (ab237709)

CD8 alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD8 alpha antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized human PBMC (peripheral blood mononuclear cells) labeling CD8 alpha with ab237709 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining in subsets of human PBMC. The nuclear counter stain is DAPI (blue). CD3 is detected with an anti-CD3 antibody-Alexa Fluor® 647 comjugate at 1/200 dilution (red).
Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

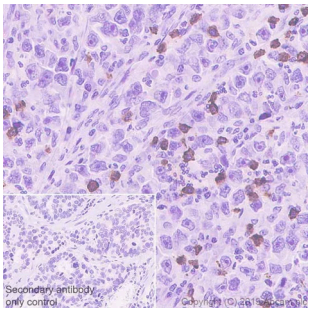


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD8 alpha antibody [CAL66] (ab237709)

CD8 alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD8 alpha antibody

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling CD8 alpha with ab237709 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Positive staining on the T lymphocytes in human colon is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

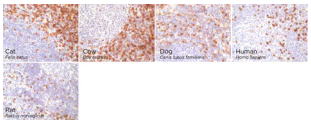
Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD8 alpha antibody [CAL66] (ab237709)

Immunohistochemical analysis of paraffin-embedded human gastric carcinoma tissue labeling CD8 alpha with ab237709 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Positive staining on the T lymphocytes in human gastric carcinoma is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

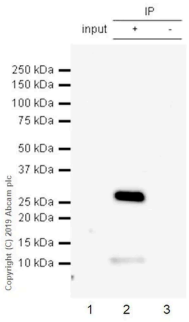


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD8 alpha antibody [CAL66] (ab237709)

CD8 alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD8 alpha antibody

The BOND™ Polymer Refine Detection System on the BOND RX Automated Stainer (DS9800, Leica Biosystems, Wetzlar, Germany) was used for immunohistochemical labelling of formalin-fixed, paraffin-embedded (FFPE) tissues. The protocol includes a peroxide block, post-primary reagent, polymer detection, DAB chromogen, and haematoxylin counterstain, all automated to minimize variability. Antigen retrieval was performed by heat-induced epitope retrieval (HIER) for 20

minutes in Tris-EDTA buffer (BOND Epitope Retrieval Solution 2, AR9640, Leica Biosystems, Wetzlar, Germany). For mouse and rabbit antibodies, the sequence comprised peroxide block (10 min), primary antibody incubation (30 min), post-primary (10 min), polymer (10 min), DAB (10 min), and haematoxylin (8 min). For rat antibodies, an anti-rat step (1:300 dilution, Vector Laboratories, California, USA; 20 min) was included prior to polymer application (15 min).



Immunoprecipitation - Anti-CD8 alpha antibody [CAL66] (ab237709)

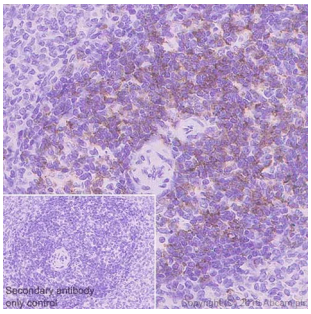
CD8 alpha was immunoprecipitated from 0.35 mg of human thymus lysate with ab237709 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab237709 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used as secondary antibody at 1/5000 dilution.
Lane 1: Human thymus lysate 10 µg (Input).
Lane 2: ab237709 IP in human thymus lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab237709 in human thymus lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 100 seconds.

All lanes:
Immunoprecipitation - Anti-CD8 alpha antibody [CAL66] (ab237709)

Predicted band size: 26 kDa

Observed band size: 26 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD8 alpha antibody [CAL66] (ab237709)

CD8 alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD8 alpha antibody

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling CD8 alpha with ab237709 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Positive staining on the rat spleen is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

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