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Product datasheet

Anti-CD8 alpha antibody [OX-8] ab33786

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Overview

Product name	Anti-CD8 alpha antibody [OX-8]
Description	Mouse monoclonal [OX-8] to CD8 alpha
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, ICC/IF, IHC-P, WB
Species reactivity	**Reacts with:** Rat
Immunogen	Tissue, cells or virus. High molecular weight rat thymocyte glycoproteins
Positive control	WB: Rat thymus tissue lysate. IHC-P: Rat Spleen. Flow Cyt: Rat splenocytes. ICC/IF: Rat splenocyte cells.
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	OX-8
Myeloma	NS1

Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab33786 in the following tested applications.

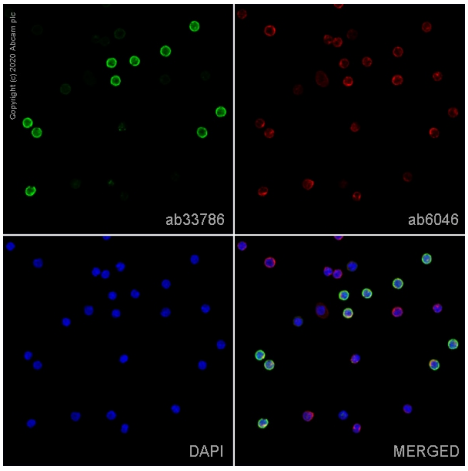
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		1/125000.
ICC/IF		Use at an assay dependent concentration.
IHC-P	★★★★★ **(2)**	Use a concentration of 5 µg/ml.
WB		Use a concentration of 1 µg/ml. Predicted molecular weight: 26 kDa.

Target

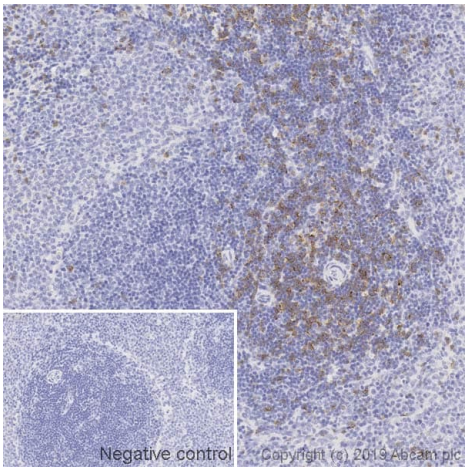
Function	Identifies cytotoxic/suppressor T-cells that interact with MHC class I bearing targets. CD8 is thought to play a role in the process of T-cell mediated killing. CD8 alpha chains binds to class I MHC molecules alpha-3 domains.
Involvement in disease	Defects in CD8A are a cause of familial CD8 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 Ig-like V-type (immunoglobulin-like) domain.
Post-translational modifications	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.

Images



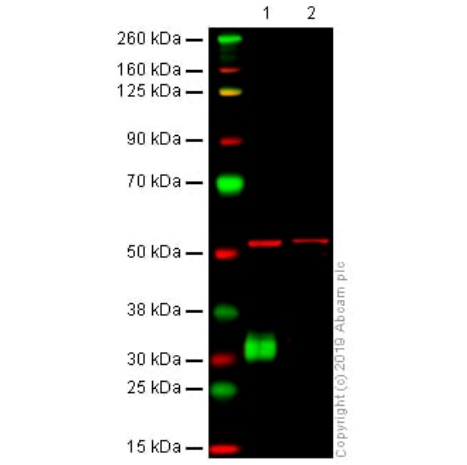
Immunocytochemistry/ Immunofluorescence - Anti-CD8 alpha antibody [OX-8] (ab33786)

ab33786 staining CD8 alpha in Rat splenocytes cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab33786 at 5µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Also suitable in cells fixed with 100% methanol (5 min).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD8 alpha antibody [OX-8] (ab33786)

IHC image of CD8 alpha staining in a section of formalin-fixed paraffin-embedded normal Rat Spleen performed on a Leica BOND™ system using the standard Protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with **ab33923**, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody. For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-CD8 alpha antibody [OX-8] (ab33786)

All lanes : Anti-CD8 alpha antibody [OX-8] (ab33786) at 1 µg/ml

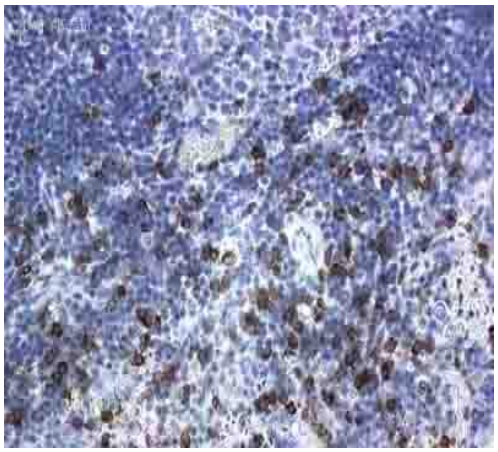
Lane 1 : Rat thymus tissue lysate
Lane 2 : Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 26 kDa
Observed band size: 32 kDa

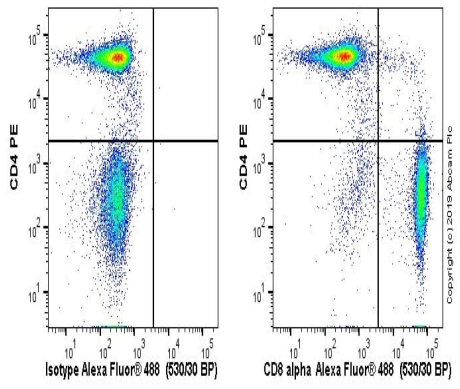
This blot was produced using a 4-12% Bis-tris under the MOPS buffer system. The gel was run at 200V for 55 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was blocked for an hour using 3% milk before ab33786 and **ab176560** (Rabbit anti-alpha Tubulin loading control) were incubated overnight at 4°C at a 1 µg/ml concentration and 1/20000 dilution respectively. Antibody binding was detected using Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD8 alpha antibody [OX-8] (ab33786)

Image courtesy of an anonymous Abreview.

ab33786 staining CD8 alpha in rat spleen tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).
Tissue was fixed with 10% neutral buffered formalin and a heat mediated antigen retrieval step was performed using citrate buffer. Samples were then permeabilized with Triton X-100, blocked using 5% serum for 30 minutes at 25°C and then incubated with ab33786 at a 1/100 dilution for 1 hour at 25°C. The secondary used was a biotin-conjugated rabbit anti-mouse polyclonal, used at a 1/300 dilution.



Flow Cytometry - Anti-CD8 alpha antibody [OX-8] (ab33786)

Flow cytometry staining of Lewis rat splenocytes with ab33786 (right) or mouse IgG1 kappa; (**ab170190**) isotype (left). Cells were incubated for 30 min on ice in 1x PBS containing 10 % rat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab33786) or mouse IgG1 kappa; (**ab170190**) isotype (1x 10^6 in 100 µl at 0.008 µg/ml) for 30 min on ice.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150177**) was used at 1/2000 dilution for 30 min on ice.

The cells were simultaneously stained with CD4.

Acquisition of >30,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. Events were gated on live CD3 positive T cells.

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