abcam

Product datasheet

Anti-CD4 antibody [EPR19514] ab183685



★★★★★ 20 Abreviews 263 References 19 Images

Overview

Product name Anti-CD4 antibody [EPR19514]

Description Rabbit monoclonal [EPR19514] to CD4

Host species Rabbit

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

Species reactivity Reacts with: Mouse

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

Positive control WB: Mouse thymus, liver, brain and kidney tissue lysates; Raw264.7 and NIH/3T3 cell lysates IHC-

P: Mouse spleen, colon, brain, cerebrum, lung and thymus tissues; mammary tumor tissue IHC-Fr:

Mouse spleen and liver tissue. IP: Mouse thymus whole cell lysate.

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

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: 7.2

Preservative: 0.01% Sodium azide

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

Purity Protein A purified

Clonality Monoclonal Clone number EPR19514

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab183685 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
IP		1/40.
IHC-P	**** (14)	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr	★★★★★ (4)	1/200.
WB	★★★★☆ (1)	1/1000. Detects a band of approximately 51 kDa (predicted molecular weight: 51 kDa).

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Function Accessory protein for MHC class-II antigen/T-cell receptor interaction. May regulate T-cell

activation. Induces the aggregation of lipid rafts.

Sequence similaritiesContains 3 lg-like C2-type (immunoglobulin-like) domains.

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

Post-translational

modifications

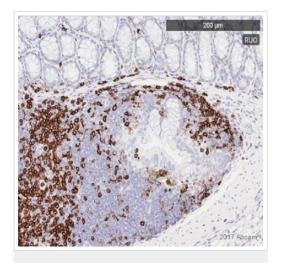
Palmitoylation and association with LCK contribute to the enrichment of CD4 in lipid rafts.

Cellular localization Cell membrane. Localizes to lipid rafts. Removed from plasma membrane by HIV-1 Nef protein

that increases clathrin-dependent endocytosis of this antigen to target it to lysosomal degradation. Cell surface expression is also down-modulated by HIV-1 Envelope polyprotein gp160 that

interacts with, and sequesters CD4 in the endoplasmic reticulum.

Images



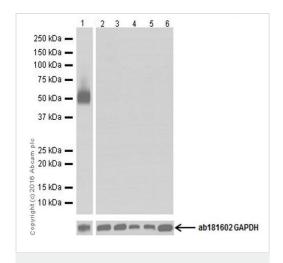
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

Verified customer

Formaldehyde-fixed, non-permeabilized mouse colon tissue stained for CD4 with ab183685 (30 mins at a 1/500 dilution) in immunohistochemical analysis. A Rabbit polyclonal HRP conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: pH 9.0 EDTA.

Blocking step: 1% ab64226 for 10 mins at RT.



Western blot - Anti-CD4 antibody [EPR19514] (ab183685)

All lanes : Anti-CD4 antibody [EPR19514] (ab183685) at 1/2000 dilution

Lane 1: Mouse thymus lysate

Lane 2 : Mouse liver tissue lysate

Lane 3 : Mouse brain tissue lysate

Lane 4: Mouse kidney tissue lysate

Lane 5: Raw264.7 (mouse abelson murine leukemia virus-induced

tumor) whole cell lysate

Lane 6: NIH/3T3 (mouse embryo) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

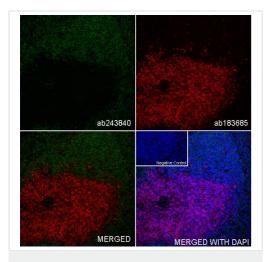
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 51 kDa **Observed band size:** 51 kDa

The expression profile observed is consistent with what has been described in the literature (PMID: 2155425).

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1:3 seconds; Lanes 2,3,4,5 and 6:30 seconds



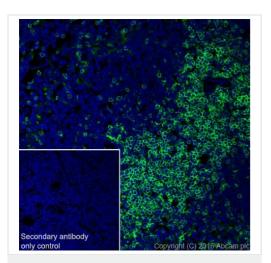
Immunohistochemistry (Frozen sections) - Anti-CD4 antibody [EPR19514] (ab183685)

IHC image of CD4 staining in a section of frozen normal Mouse Spleen.

The section was fixed using 10% formaldehyde in 1XPBS for 10 minutes. No antigen retrieval step was performed prior to staining. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M 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 ab183685 (Rabbit monoclonal [EPR19514] to CD4) at 1/200 and ab243840 at 1µg/ml, to show the distinct staining of B cells and T cells. The section was then incubated with ab150165 (Goat Anti-Rat lgG H&L (Alexa Fluor® 488) preabsorbed, (Shown in green) 1/1000) and ab150080 (Goat Anti-Rabbit lgG H&L (Alexa Fluor®594) (Shown in red) 1/1000) for 1 hour at room temperature. The secondary-only control insert image is taken from an identical assay without primary antibody. DAPI was used to stain the cell nuclei (blue). The section was then mounted using Fluoromount®.

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

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



Immunohistochemistry (Frozen sections) - Anti-CD4 antibody [EPR19514] (ab183685)

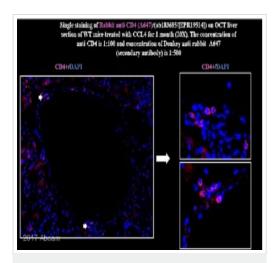
Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen Mouse spleen tissue labeling CD4 with ab183685 at 1/500 dilution, followed by Goat Anti-Rabbit lgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green).

The result showed membrane staining on mouse spleen.

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab150077</u> at 1/1000 dilution.

Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20).

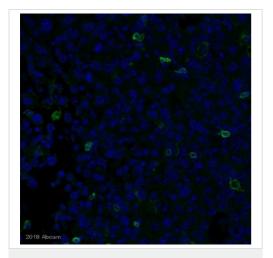


Immunohistochemistry (Frozen sections) - Anti-CD4 antibody [EPR19514] (ab183685)

Verified customer

Paraformaldehyde-fixed, 0.1% Tween 0.3% Triton in PBS permeabilized mouse liver tissue stained for CD4 with ab183685 (12 hours, $4^{\circ}C$ at a 1/100 dilution) in immunohistochemical analysis. A Donkey anti Rabbit IgG (H+L) AlexaFluor $^{\!8}\!647$ was used as the secondary at a 1/500 dilution (red).

Blocking step: 1% BSA for 12 hours at 4°C.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

Anugraha Rajagopalan

Formalin-fixed, 0.2% Triton-X permeabilized mouse Eo771 mammary tumor tissue stained for CD4 with ab183685 (18 hours, 4°C at a 1/1000 dilution) in immunohistochemical analysis. A Goat anti Rabbit lgG (H&L) polyclonal AlexaFluor[®]488 conjugate was used as the secondary (green).

Heat mediated antigen retrieval buffer/enzyme used: Citrate buffer. Blocking step: 4% BSA for 1 hour at 25°C.



Formaldehyde-fixed, non-permeabilized mouse brain tissue stained for CD4 with ab183685 (14 hours, 4°C at a 1/1000 dilution) in immunohistochemical analysis. A Goat anti Rabbit lgG monoclonal HRP conjugate was used as the secondary at a 1/200 dilution.

Blocking step: 5% normal goat serum for 1 hour at 26°C.

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

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

Herr Dr. Markus Kipp

2018 Aboam

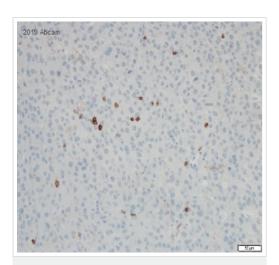
Formaldehyde-fixed, paraffin-embedded mouse lung tissue stained for CD4 with ab183685 (18 hours, 4°C at a 1/1000 dilution) in immunohistochemical analysis. A Goat anti Rabbit lgG polyclonal HRP conjugate was used as the secondary at a 1/250 dilution (red).

Heat mediated antigen retrieval buffer/enzyme used: Citrate buffer pH 6.0.

Blocking step: 10% serum for 1 hour at 25°C.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

Verified customer

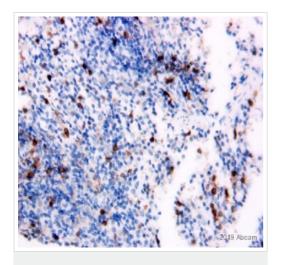


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

Verified customer

Formaldehyde-fixed, 3% hydrogen peroxide permeabilized B16F10 (melanoma) syngeneic mouse tumor tissue stained for CD4 with ab183685 (36mins, 37°C at a 1/1000 dilution) in immunohistochemical analysis. A Goat anti Rabbit lgG polyclonal HRP conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: Tris/EDTA.

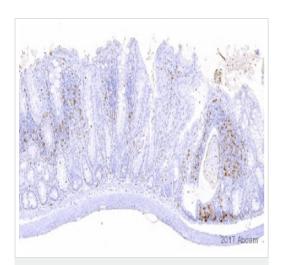


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

Zohar Gavish

Formaldehyde-fixed, non-permeabilized mouse spleen tissue stained for CD4 with ab183685 (30mins at a 1/1000 dilution) in immunohistochemical analysis. A Goat polyclonal HRP conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: Leica ER2 solution



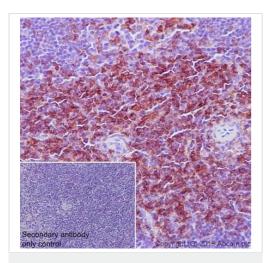
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

Verified customer

Formaldehyde-fixed mouse colon, chronic mucosal inflammation tissue stained for CD4 with ab183685 (90 mins at a 1/1000 dilution) in immunohistochemical analysis. A Goat polyclonal biotin conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: High pH CC1.

Blocking step: 1% BSA for 40 mins at 24°C.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

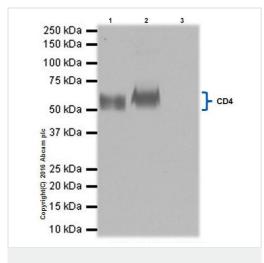
Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling CD4 with ab183685 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Membrane staining on T cells is observed.

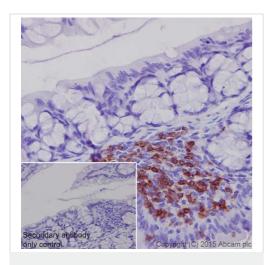
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution.

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



Immunoprecipitation - Anti-CD4 antibody [EPR19514] (ab183685)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

CD4 was immunoprecipitated from 1mg of Mouse thymus whole cell lysate with ab183685 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab183685 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

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

Lane 2: ab183685 IP in Mouse thymus whole cell lysate.

Lane 3: Rabbit lgG,monoclonal [EPR25A] - Isotype Control (ab172730) instead of ab183685 in Mouse thymus whole cell lysate.

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

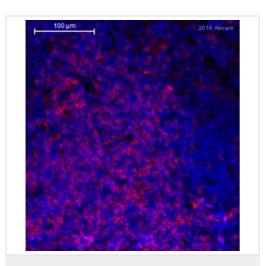
Exposure time: 5 seconds.

Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labeling CD4 with ab183685 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Membrane staining on lymphocytes and negative on epithelium cells of mouse colon is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution.

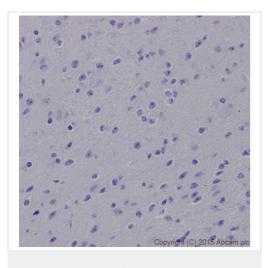
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



10% NBF-fixed, paraffin-embedded mouse spleen tissue stained for CD4 using ab183685 at 1/2000 dilution in immunohistochemical analysis, followed by Goat anti-Rabbit IgG Alexa Fluor[®] 647.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

This image is courtesy of an anonymous Abreview.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

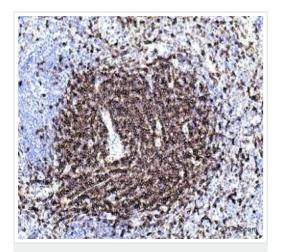
Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling CD4 with ab183685 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Negative on mouse cerebrum.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

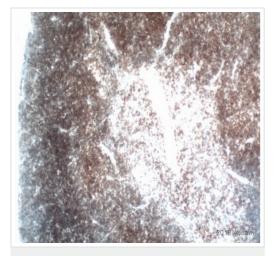
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Formaldehyde-fixed, paraffin-embedded mouse spleen tissue stained for CD4 using ab183685 at 1/2000 dilution in immunohistochemical analysis, followed by Goat anti Rabbit lgG Biotin.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

This image is courtesy of an Abreview submitted by Mr . Carl Hobbs.



Formaldehyde-fixed, paraffin-embedded mouse thymus tissue stained for CD4 using ab183685 at 1/500 dilution in immunohistochemical analysis.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody
[EPR19514] (ab183685)

This image is courtesy of an anonymous Abreview.



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