## abcam

#### Product datasheet

### Anti-CD4 antibody [EPR6855] ab133616



重组 RabMAb

★★★★★ 18 Abreviews 242 References 21 图像

概述

产品名称 Anti-CD4抗体[EPR6855]

描述 兔单克隆抗体[EPR6855] to CD4

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), mIHC, WB, IHC-P, ICC/IF

种属反应性 与反应: Human

不与反应: Mouse. Rat

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

阳性对照 WB: THP-1 and HuT-78 cell lysates, human fetal thymus, tonsil and lymph node tissue lysates.

> IHC-P: Human tonsil, liver, spleen, thymoma and colon tissues. ICC/IF: Human peripheral blood lymphocytes and THP-1 cells. Flow Cyt (intra): Human peripheral blood lymphocytes. mlHC: Hu

lung cancer tissue

This product is a recombinant monoclonal antibody, which offers several advantages including: 常规说明

- 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**.

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Stable for 12 months at -20°C.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol, PBS

纯度 Protein A purified

克隆 单克隆

**克隆编号** EPR6855

**同种型** IgG

#### 应用

#### The Abpromise guarantee Abpromise™承诺保证使用ab133616于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/100.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
mIHC		1/600.
WB		1/5000. Detects a band of approximately 51 kDa (predicted molecular weight: 51 kDa).  For unpurified use at 1/1000 - 1/10000.
IHC-P	<b>★★★★★ (14)</b>	1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.  For unpurified use at 1/100 - 1/250.
ICC/IF	<b>★★★★★</b> (2)	1/100 - 1/250.

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

activation. Induces the aggregation of lipid rafts.

序列相似性 Contains 3 lg-like C2-type (immunoglobulin-like) domains.

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

翻译后修饰 Palmitoylation and association with LCK contribute to the enrichment of CD4 in lipid rafts.

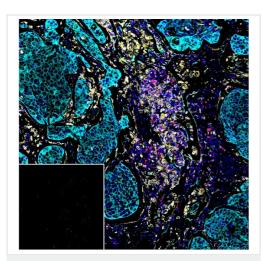
细胞定位 Cell membrane. Localizes to lipid rafts. Removed from plasma membrane by HIV-1 Nef protein

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.

#### 图片



Multiplex immunohistochemistry - Anti-CD4 antibody [EPR6855] (ab133616)

This image is courtesy of TissueGnostics Asia Pacific Limited

10-color fluorescence multiplex immunohistochemical analysis of human lung cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of anti-FOXP3 (ab215206; Cyan; TG540N), anti-PD1 (ab52587; Red; TG700N), anti-CD163 (ab182422; Brown; TG650N), anti-HLA-DR (ab92511; Yellow; TG570N), anti-CD4 (ab133616; Violet; TG620N), anti-CD8 alpha (ab101500; Purple; TG540S), anti-CD20 (ab9475; Grey; TG660S), anti-CD68 (ab192847; Green; TG520N), anti-Cytokeratin 19 (ab52625; Light blue; TG440N). TG470SN (dark blue) was used as a nuclear counter stain. The inset image shows the separate CD4 signal.

The section was incubated in nine rounds of staining; in the order of <a href="mailto:ab215206">ab215206</a> (1/100 dilution), <a href="mailto:ab52587">ab52587</a> (1/200 dilution), <a href="mailto:ab133616">ab133616</a> (1/600 dilution), <a href="mailto:ab92511">ab92511</a> (1/200 dilution), <a href="mailto:ab9475">ab133616</a> (1/600 dilution), <a href="mailto:ab9475">ab192847</a> (1/300 dilution), <a href="mailto:ab52625">ab52625</a> (1/400 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (pH6.0) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

Image acquisition was performed with TissueFAXS Spectra (TissueGnostics).

Multiplex immunohistochemistry - Anti-CD4 antibody [EPR6855] (ab133616)

This image is courtesy of TissueGnostics Asia Pacific Limited

10-color fluorescence multiplex immunohistochemical analysis of human lung cancer tissue (formalin-fixed paraffin-embedded section).

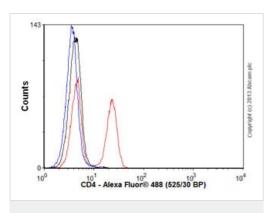
Merged staining of anti-FOXP3 (<u>ab215206</u>; Cyan; TG540N), anti-PD1 (<u>ab52587</u>; Violet; TG700N), anti-CD163 (<u>ab182422</u>; Red; TG650N), anti-HLA-DR (<u>ab92511</u>; Yellow; TG570N), anti-CD4 (<u>ab133616</u>; Orange; TG620N), anti-CD8 alpha (<u>ab101500</u>; Purple; TG540S), anti-CD20 (<u>ab9475</u>; Grey; TG660S), anti-CD68 (<u>ab192847</u>; Green; TG520N), anti-Cytokeratin 19 (<u>ab52625</u>; Light blue; TG440N). TG470SN (dark blue) was used as a nuclear counter stain.

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<u>ab192847</u> (1/300 dilution), <u>ab52625</u> (1/400 dilution); each using a separate fluorescent tyramide signal amplification system.

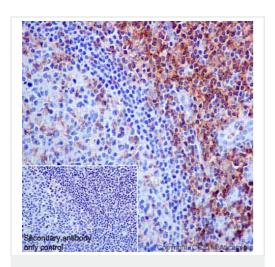
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Image acquisition was performed with TissueFAXS Spectra (TissueGnostics).



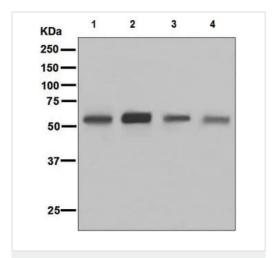
Flow Cytometry (Intracellular) - Anti-CD4 antibody [EPR6855] (ab133616)

Human peripheral blood lymphocytes stained with unpurifiedab133616 (red line). Human whole blood was processed using a modified protocol based on Chow et al, 2005 (PMID: 16080188). In brief, human whole blood was fixed in 4% formaldehyde (methanol-free) for 10 min at 22°C. Red blood cells were then lyzed by the addition of Triton X-100 (final concentration -0.1%) for 15 min at 37°C. For experimentation, cells were treated with 50% methanol (-20°C) for 15 min at 4°C. Cells were then incubated with the antibody (unpurified ab133616, 1/100 dilution) for 30 min at 4°C. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit lgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 4°C. Isotype control antibody (black line) was rabbit IgG (monoclonal)  $(0.1 \mu g/1 x 10^6 \text{ cells})$  used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >30,000 total events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. Gating strategy - peripheral blood lymphocytes.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling CD4 with purified ab133616 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-CD4 antibody [EPR6855] (ab133616)

**All lanes :** Anti-CD4 antibody [EPR6855] (ab133616) at 1/1000 dilution (unpurified)

Lane 1: THP-1 cell lysate

Lane 2: Human fetal thymus lysate

Lane 3: Human tonsil lysate

Lane 4: Human lymph node lysate

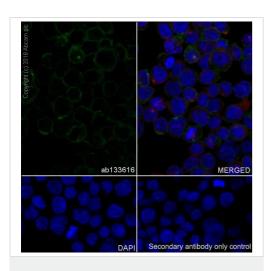
Lysates/proteins at 10 µg per lane.

#### **Secondary**

Lane 1: HRP labelled goat anti-rabbit at 1/2000 dilution

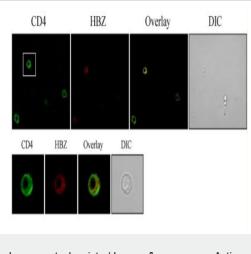
Lanes 2-4: HRP labelled goat anti-rabbit at 1/2000 dilution

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



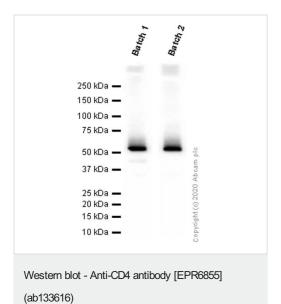
Immunocytochemistry/ Immunofluorescence - Anti-CD4 antibody [EPR6855] (ab133616)

Immunocytochemistry analysis of THP-1 (Human monocytic leukemia monocyte) labeling CD4 with purified ab133616 at 1/100 dilution. Cells were fixed with 100% methanol. Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) at 1/1000 (2 μg/ml) was used as the secondary antibody. **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.30 μg/ml) was used as counterstain. Nuclei were stained blue with DAPI. Negative control: PBS instead of the primary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-CD4 antibody [EPR6855] (ab133616)

Baratella et al PLoS Negl Trop Dis. 2017 Jan; 11(1): e0005285. Published online 2017 Jan 17. doi: 10.1371/journal.pntd.0005285



# HBZ is preferentially expressed in CD4+ T cells of HAM/TSP patient PH1624

Confocal microscopy analysis of PBMC from HAM/TSP patient PH1624. (A) co-staining with the 4D4-F3 anti-HBZ mAb followed by Alexa Fluor 546-conjugated goat anti-mouse IgG1 antibody (red) and with the anti-CD4 mAb followed by Alexa Fluor 488-conjugated goat-anti-rabbit IgG antibody (green); upper panels, extended field; lower panels, enlarged field focused on the single cell depicted in the square of the left upper panel and positive for both CD4 and HBZ.

CD4 was detected using ab133616 at 1/100 dilution.

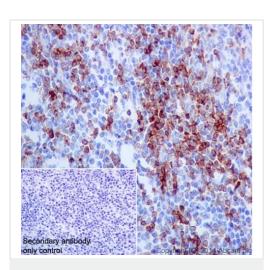
From Figure 6A of Baratella et al.

Baratelle et al <u>PLoS Negl Trop Dis</u>. 2017 Jan; 11(1): e0005285. Published online 2017 Jan

17. doi: 10.1371/journal.pntd.0005285

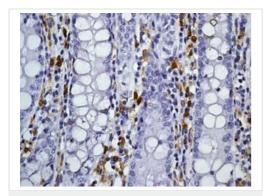
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Different batches of ab133616 were tested on THP-1 (Human monocytic leukemia monocyte) lysate at 1.0  $\mu$ g/ml. 15  $\mu$ g of lysate was loaded in each lane. Bands observed at 51 kDa.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

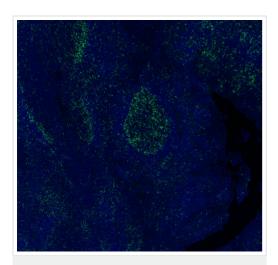
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thymoma tissue labelling CD4 with purified ab133616 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

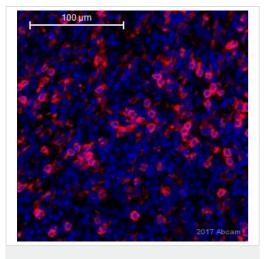
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue labelling CD4 with ab133616 at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Anti-CD4 antibody [EPR6855] (ab133616)
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling CD4 with ab133616 at a dilution of 1:500. Heat mediated antigen retrieval was performed using AR9 antigen retrieval solution, and microwave treatment for 15 min at 20% power. Anti-Rabbit/Mouse HRP polymer (PerkinElmer Opal Polymer HRP Ms Plus Rb) was used as secondary antibody. Opal tyramide amplification was performed using Opal 520 fluorophore. Counterstained with DAPI stain. Image scanned with Vectra 3.0 and analyzed via Phenochart software. This image was courteously provided by Dr. Houssein Abdul Sater,

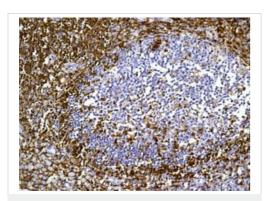


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

This image is courtesy of an anonymous Abreview.

Paraffin-embedded human spleen tissue stained for CD4 using ab133616 at 1/500 dilution in immunohistochemical analysis.

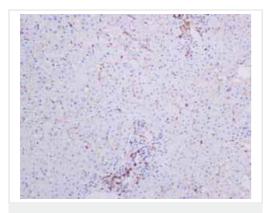
Georgia Cancer Center.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling CD4 with unpurified ab133616 at a dilution of 1/100.

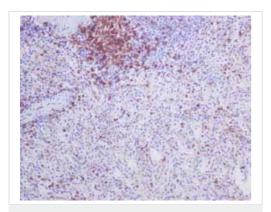
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling CD4 with unpurified ab133616.

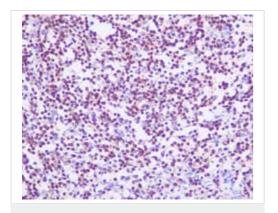
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human spleen tissue labelling CD4 with unpurified ab133616.

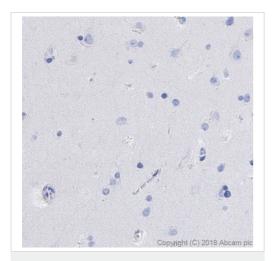
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thymoma tissue labelling CD4 with unpurified ab133616.

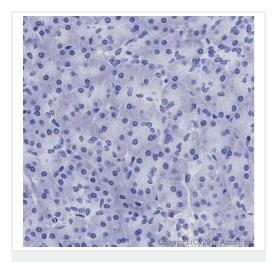
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Negative control: no staining on human cerebrum.

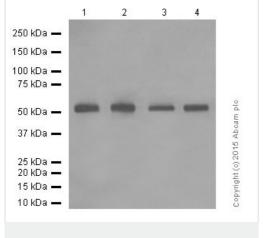
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebrum showing no staining CD4 with purified ab133616 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9 (ab93684). Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Negative control: no staining on human pancreas.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human pancreas showing no staining CD4 with purified ab133616 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9 (ab93684). Goat Anti-Rabbit lgG H&L (HRP) was used as the secondary antibody. Counterstained with hematoxylin.



Western blot - Anti-CD4 antibody [EPR6855] (ab133616)

**All lanes :** Anti-CD4 antibody [EPR6855] (ab133616) at 1/5000 dilution (purified)

Lane 1: Human fetal thymus tissue lysate

Lane 2: Human tonsil tissue lysate

Lane 3 : THP-1 cell lysate

Lane 4 : HuT-78 cell lysate

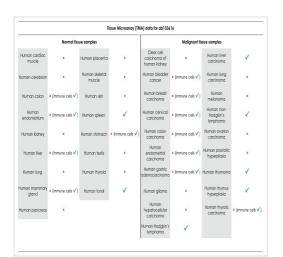
Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG at 1/1000 dilution

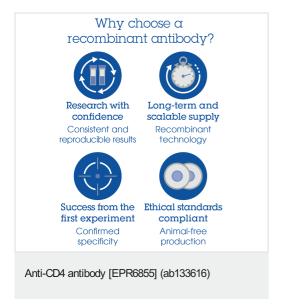
**Predicted band size:** 51 kDa **Observed band size:** 51 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Tissue Microarrays stained for "Anti-CD4 antibody [EPR6855]" using "ab133616" 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 ab133616 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.



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