Product datasheet

Anti-CD4 antibody [EPR6855] ab133616

Overview

Product name
Anti-CD4 antibody [EPR6855]

Description
Rabbit monoclonal [EPR6855] to CD4

Host species
Rabbit

Tested applications
Suitable for: WB, IHC-P, Flow Cyt, ICC/IF

Species reactivity
Reacts with: Human
Does not react with: Mouse, Rat

Immunogen
Recombinant fragment within Human CD4 aa 200-400. The exact sequence is proprietary.
Database link: P01730

Positive control
WB: THP-1 and HuT-78 cell lysates, human fetal thymus, tonsil and lymph node tissue lysates.

General notes
Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

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. Stable for 12 months at -20°C.

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 0.05% BSA, 40% Glycerol, PBS
Purity: Protein A purified
Clonality: Monoclonal
Clone number: EPR6855
Isotype: IgG

Function:
Accessory protein for MHC class-II antigen/T-cell receptor interaction. May regulate T-cell activation. Induces the aggregation of lipid rafts.

Sequence similarities:
Contains 3 Ig-like C2-type (immunoglobulin-like) domains.
Contains 1 Ig-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.

Applications:
Our Abpromise guarantee covers the use of ab133616 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>1/5000. Detects a band of approximately 51 kDa (predicted molecular weight: 51 kDa). For unpurified use at 1/1000 - 1/10000.</td>
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<tr>
<td>IHC-P</td>
<td>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.</td>
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<tr>
<td>Flow Cyt</td>
<td>1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>ICC/IF</td>
<td>1/100 - 1/250.</td>
<td></td>
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</tbody>
</table>

Target:

Function:
Accessory protein for MHC class-II antigen/T-cell receptor interaction. May regulate T-cell activation. Induces the aggregation of lipid rafts.

Images
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. ab97051, 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.

Human peripheral blood lymphocytes stained with unpurified ab133616 (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 lysed 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 Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 4°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1μg/1x10^6 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 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. ab97051, 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.

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

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

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

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

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

**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 IgG 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 1/1000 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

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

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**Please note**: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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