Product name: Anti-CD4 antibody [EPR6855] ab133616

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


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.

Overview

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

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>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td></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>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★★</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>
</tr>
<tr>
<td>Flow Cyt</td>
<td>1/100</td>
<td>ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>1/100 - 1/250.</td>
</tr>
</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.
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.

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

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/1x10⁶ 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) - 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. 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.
**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 IgG, specific to the non-reduced form of IgG 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 paraffin-embedded 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. 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.
**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

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

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

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**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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