# abcam

## Product datasheet

# Anti-CD4 antibody [CAL4] ab237722

Recombinant RabMAb

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Overview

**Product name** Anti-CD4 antibody [CAL4]

**Description** Rabbit monoclonal [CAL4] to CD4

**Host species** Rabbit

**Tested applications** Suitable for: WB, IHC-Fr, IP, Flow Cyt (Intra), IHC-P

Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse. Rat

**Immunogen** Synthetic peptide within Mouse CD4 aa 417-457. The exact sequence is proprietary.

Database link: P06332

Positive control IHC-P: Mouse and rat spleen tissue. WB: Mouse and rat thymus, spleen and lymph node lysate.

IHC-Fr: Mouse spleen tissue. Flow Cyt (intra): Mouse splenocytes. IP: Mouse thymus lysate.

**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: 0.05% Sodium azide

Constituent: PBS

**Purity** Protein A purified

**Purification notes** Purity >99%. Clonality Monoclonal

Clone number CAL4

Isotype ΙgG

**Applications** 

Our **Abpromise guarantee** covers the use of ab237722 in the following tested applications. The Abpromise guarantee

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

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Application	Abreviews	Notes
WB		1/1000. Predicted molecular weight: 51 kDa.
IHC-Fr		1/100. Perform heat mediated antigen retrieval using sodium citrate buffer (10 mM citrate pH 6.0 and 0.05% Tween-20).
IP		1/30.
Flow Cyt (Intra)		1/40.
IHC-P	****(1)	1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

**Application notes** 

Is unsuitable for ICC/IF.

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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 similarities**Contains 3 lg-like C2-type (immunoglobulin-like) domains.

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

Post-translational

modifications

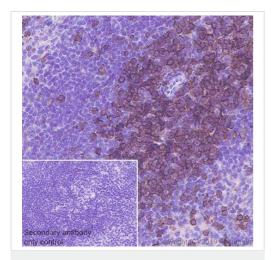
Palmitoylation and association with LCK contribute to the enrichment of CD4 in lipid rafts.

**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

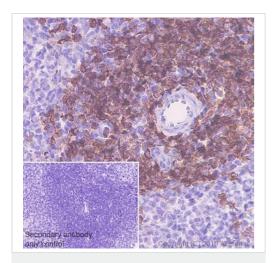
interacts with, and sequesters CD4 in the endoplasmic reticulum.

#### **Images**



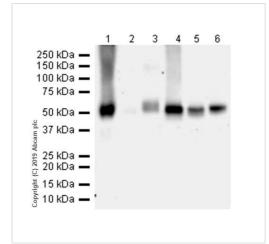
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [CAL4] (ab237722)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling CD4 with ab237722 at 1/4000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse spleen. The section was incubated with ab237722 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [CAL4] (ab237722)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling CD4 with ab237722 at 1/4000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on rat spleen. The section was incubated with ab237722 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



Western blot - Anti-CD4 antibody [CAL4] (ab237722)

**All lanes :** Anti-CD4 antibody [CAL4] (ab237722) at 1/1000 dilution

Lane 1: Mouse thymus lysate

Lane 2: Mouse spleen lysate

Lane 3: Mouse lymph node lysate

Lane 4: Rat thymus lysate

Lane 5: Rat spleen lysate

Lane 6: Rat lymph node lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit  $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$  at

1/100000 dilution

Predicted band size: 51 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Secondary antibody
only control

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Immunohistochemistry (Frozen sections) - Anti-CD4 antibody [CAL4] (ab237722)

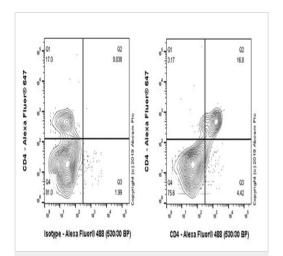
Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse spleen tissue labeling CD4 with ab237722 at 1/100 dilution (green), followed by <u>ab150077</u>

AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary at a 1/1,000 dilution.

Positive staining on T cell surface (PMID: 26994219) is observed.

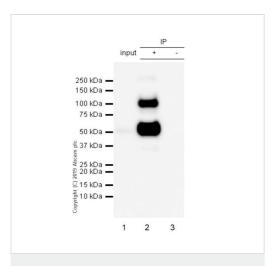
Counterstained with DAPI (blue). Secondary antibody only control:

Used PBS instead of primary antibody, secondary antibody is <u>ab150077</u> AlexaFluor<sup>®</sup>488 Goat anti-Rabbit used at a 1/1,000 dilution. Heat mediated antigen retrieval using sodium citrate buffer (10 mM citrate pH 6.0 and 0.05% Tween-20).



Flow Cytometry (Intracellular) - Anti-CD4 antibody [CAL4] (ab237722)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized mouse splenocytes labeling CD4 with ab237722 at 1/40 (right panel) compared with a Rabbit monoclonal IgG (ab172730) (left panel). Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, ab150077), at 1/2000 dilution was used as the secondary antibody. Cells were surface stained with anti-CD4 conjugated to Alexa Fluor<sup>®</sup> 647. Then fixed with 2% PFA for 10min and intracellularly stained with rabbit IgG (Left) or ab237722 (Right). They recognize the same cell population.



Immunoprecipitation - Anti-CD4 antibody [CAL4] (ab237722)

CD4 was immunoprecipitated from 0.35 mg mouse thymus lysate with ab237722 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab237722 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used at 1/5000 dilution.

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

Lane 2: ab237722 IP in mouse thymus lysate .

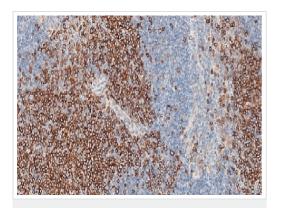
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab237722 in

mouse thymus lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

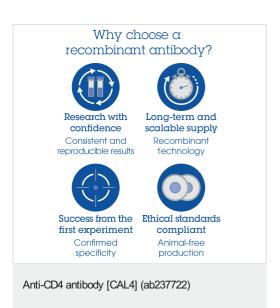
Exposure time: 30 seconds.

102kDa band represents CD4 dimer based on literature. (PMID: 20739067).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [CAL4] (ab237722)

Formalin-fixed, paraffin-embedded mouse spleen tissue stained for CD4 using ab237722 at 0.5  $\mu$ g/ml in immunohistochemical analysis.



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