abcam

ab213215

# Anti-CD4 antibody [SP35]

Anti-CD4 antibody [SP35] (ab213215) is a rabbit monoclonal antibody detecting CD4 in **Flow Cytometry, IHC-P, ICC/IF, mIHC**. Suitable for **Human**.

- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency
- Over 10 publications

Lab Essentials | Recombinant | RabMAb | Advanced Validation | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.6 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | SP35 |
| **Purification technique** | Affinity purification Protein A/G |
| **Concentration** | 0.108 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |
| **Purification notes** | Purified from TCS by protein A/G. |

## Reactivity data

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/100 |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Chimpanzee |
| **Dilution info** | - |
| **Notes** | - |

## mIHC

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

### Predicted

| | |
|---|---|
| **Species** | Chimpanzee |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/20 - 1/100 |
| **Notes** | Incubate with primary antibody for 30 minutes at 4°C. |

### Predicted

| | |
|---|---|
| **Species** | Chimpanzee |

| | |
|---|---|
| **Dilution info** | - |
| **Notes** | - |

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/50 |
| **Notes** | Deparaffinize slides using xylene or xylene alternative and graded alcohols. Boil tissue section in EDTA buffer for 10 min followed by cooling at room temperature for 20 min. Incubate with primary antibody for 30 minutes at room temperature. Slides must be washed in between steps. Rinse slides with PBS/0.05% Tween. |

### Predicted

| | |
|---|---|
| **Species** | Chimpanzee |
| **Dilution info** | - |
| **Notes** | - |

# Target data

See full target information CD4

**Function**

Integral membrane glycoprotein that plays an essential role in the immune response and serves multiple functions in responses against both external and internal offenses. In T-cells, functions primarily as a coreceptor for MHC class II molecule:peptide complex. The antigens presented by class II peptides are derived from extracellular proteins while class I peptides are derived from cytosolic proteins. Interacts simultaneously with the T-cell receptor (TCR) and the MHC class II presented by antigen presenting cells (APCs). In turn, recruits the Src kinase LCK to the vicinity of the TCR-CD3 complex. LCK then initiates different intracellular signaling pathways by phosphorylating various substrates ultimately leading to lymphokine production, motility, adhesion and activation of T-helper cells. In other cells such as macrophages or NK cells, plays a role in differentiation/activation, cytokine expression and cell migration in a TCR/LCK-independent pathway. Participates in the development of T-helper cells in the thymus and triggers the differentiation of monocytes into functional mature macrophages. (Microbial infection) Primary receptor for human immunodeficiency virus-1 (HIV-1) (PubMed:12089508, PubMed:16331979, PubMed:2214026, PubMed:9641677). Down-regulated by HIV-1 Vpu (PubMed:17346169). Acts as a receptor for Human Herpes virus 7/HHV-7 (PubMed:7909607).

## Storage

| | |
|---|---|
| **Shipped at conditions** | Conditional Ambient |
| **Appropriate short-term storage duration** | 1-2 weeks |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

**What is this antibody validated in?**
Anti-CD4 antibody [SP35] (ab213215) is a rabbit recombinant monoclonal antibody and is validated for use in Flow Cytometry (Flow Cyt), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), Multiplex IHC (mIHC) in Human samples.

**Collaboration**
Anti-CD4 [SP35] (ab213215) is a clone from the portfolio of Spring Bioscience (Roche) SP clones which have been optimised for immunohistochemistry (IHC).

**Trusted by the scientific community**
Anti-CD4 [SP35] (ab213215) was first used in a scientific publication in 2016 and has been cited over 10 times in peer-reviewed journals.

**Other related products**
We have a range of other formats of antibody clone [SP35] also available for your convenience: ab101530, ab213215, Carrier free - ab238798, ab279480, PE - ab305622, APC - ab305623, HRP - ab305624, Alkaline Phosphatase - ab308987, Alexa Fluor® 488 - ab309931, Alexa Fluor® 647 - ab310298, Alexa Fluor® 594 - ab310760, Alexa Fluor® 555 - ab312287, Alexa Fluor® 568 - ab312782, Alexa Fluor® 750 - ab321373

**Trial sizes available!**
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

We do not recommend this combination. It is not covered by our product promise.

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We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

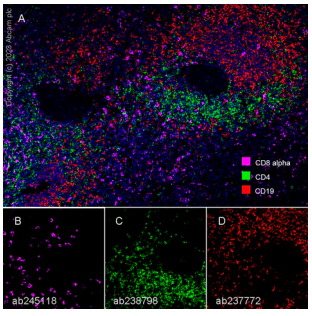
In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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## 11 product images

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### Multiplex immunohistochemistry - Anti-CD4 antibody [SP35] (ab213215)

CD4 Multiplex immunohistochemistry staining of Human spleen using rabbit Anti-CD4 antibody

This data was developed using ab238798 , the same antibody clone in a different buffer formulation.
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Human spleen tissue labeling CD8 alpha with ab245118 at 1/500 dilution, CD4 with ab238798 at 1/500, and CD19 with ab237772 at 1/5000 dilution.

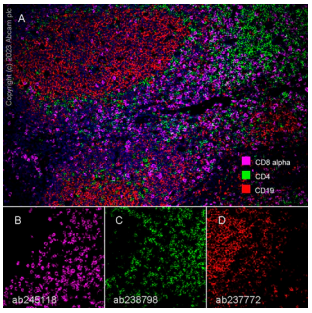
Panel A: merged staining of anti-CD8 alpha (magenta; Opal™690), anti-CD4 (green; Opal™520) and anti-CD19 (red; Opal™570) on human spleen.
Panel B: anti-CD8 alpha stained on cytotoxic T cells.
Panel C: anti-CD4 stained on T helper cells.
Panel D: anti-CD19 stained on B cells.

Sections were treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins before antibody incubation. The section was incubated in three rounds of staining: in the order of ab245118 for 30 mins, then ab238798 and ab237772 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

DAPI was used as a nuclear counterstain.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

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## Multiplex immunohistochemistry - Anti-CD4 antibody [SP35] (ab213215)

CD4 Multiplex immunohistochemistry staining of Human tonsil using rabbit Anti-CD4 antibody

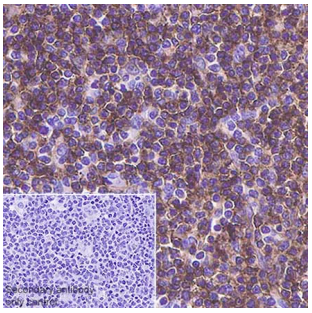
This data was developed using ab238798 , the same antibody clone in a different buffer formulation.
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Human tonsil tissue labeling CD8 alpha with ab245118 at 1/500 dilution, CD4 with ab238798 at 1/500, and CD19 with ab237772 at 1/5000 dilution.

Panel A: merged staining of anti-CD8 alpha (magenta; Opal™690), anti-CD4 (green; Opal™520) and anti-CD19 (red; Opal™570) on human tonsil.
Panel B: anti-CD8 alpha stained on cytotoxic T cells.
Panel C: anti-CD4 stained on T helper cells.
Panel D: anti-CD19 stained on B cells.

Sections were treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins before antibody incubation. The section was incubated in three rounds of staining: in the order of ab245118 for 30 mins, then ab238798 and ab237772 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

DAPI was used as a nuclear counterstain.

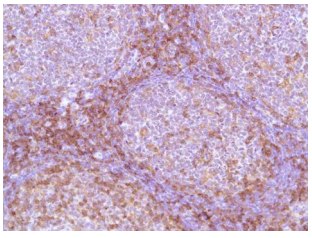
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD4 antibody [SP35] (ab213215)

CD4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD4 antibody

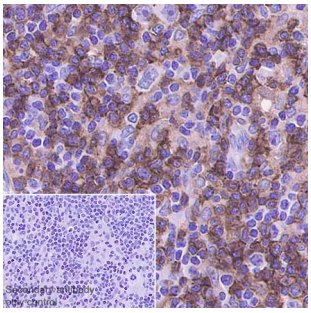
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil tissue sections labeling CD4 with ab213215 at 1/50 dilution (3.1 µg/ml). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Hematoxylin was used as a counterstain. Positive staining on the human tonsil, performed on a Leica Biosystems BOND™ RX instrument.
The section was incubated with ab213215 for 30 mins at room temperature.



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD4 antibody [SP35] (ab213215)

CD4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD4 antibody

Immunohistochemical analysis of formalin-fixed, paraffin-embedded human tonsil tissue labeling CD4 with ab213215 at 1/50 dilution.

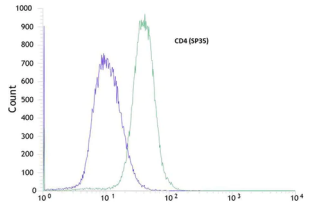


## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD4 antibody [SP35] (ab213215)

CD4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD4 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human Hodgkin lymphoma tissue sections labeling CD4 with ab213215 at 1/50 dilution (3.1 µg/ml). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Hematoxylin was used as a counterstain. Positive staining on the human Hodgkin's lymphoma, performed on a Leica Biosystems BOND™ RX instrument.
The section was incubated with ab213215 for 30 mins at room temperature.

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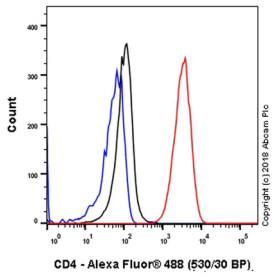



## Flow Cytometry - Anti-CD4 antibody [SP35] (ab213215)

CD4 Flow Cytometry staining using rabbit Anti-CD4 antibody

Flow cytometric analysis of Jurkat (Human T cell leukemia cell line from peripheral blood) cells labeling CD4 with ab213215 at 1/100 dilution (green), compared with a rabbit IgG negative control (blue).

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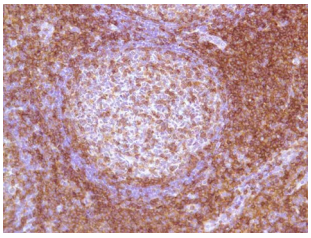



## Flow Cytometry - Anti-CD4 antibody [SP35] (ab213215)

CD4 Flow Cytometry staining using rabbit Anti-CD4 antibody

**Flow cytometry analysis of THP-1 (human acute monocytic leukemia) labeling CD4 with purified ab213215 at 1/20 dilution (7.75 µg/ml) (red).**
Goat anti rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as a secondary antibody. Isotype control - Rabbit monoclonal IgG (ab172730) (Black). Unlableled control - Unlabeled cells (blue).

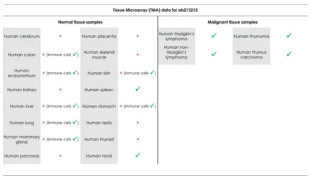
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## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD4 antibody [SP35] (ab213215)

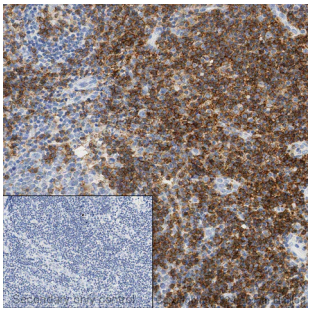
CD4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD4 antibody

Immunohistochemical analysis of formalin-fixed, paraffin-embedded human tonsil tissue labeling CD4 with ab213215 at 1/50 dilution.



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD4 antibody [SP35] (ab213215)

Tissue Microarrays stained for "Anti-CD4 antibody [SP35]" using "ab213215"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 10 minutes. The sections were incubated with ab213215 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.



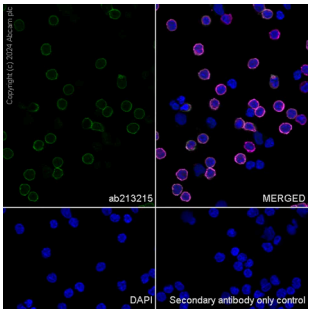

## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD4 antibody [SP35] (ab213215)

CD4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human tonsil using rabbit Anti-CD4 antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling CD4 with ab213215 at a dilution of 1/50. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

Anti-CD4 antibody [SP35] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual).




## Immunocytochemistry/ Immunofluorescence - Anti-CD4 antibody [SP35] (ab213215)

Immunocytochemistry/ Immunofluorescence analysis of human PBMC (human primary peripheral blood mononuclear cell) labeling CD4 with ab238798 at 1/100 dilution followed by ab150081 at 1/1000 dilution (green). The counterstain was Anti-human CD3 mouse monoclonal antibody (Alexa Fluor® 64) at 1/200 dilution observed in magenta. Nuclear DNA was labeled with DAPI (shown in blue). Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% Tween-20. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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