

## Product datasheet


# Anti-CD3 antibody [SP7] ab16669

RabMAb<sup>®</sup>

★★★★★ 59 Abreviews 130 References 13 Images

### Overview

---

<b>Product name</b>	Anti-CD3 antibody [SP7]
<b>Description</b>	Rabbit monoclonal [SP7] to CD3
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt, IHC-Fr, IHC-P, WB, IHC-FoFr
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human, Pig <b>Predicted to work with:</b> Sheep, Rabbit, Horse, Chicken, Cow, Cat, Dog, Baboon, Woodchuck 
<b>Immunogen</b>	Synthetic peptide corresponding to CD3.
<b>Epitope</b>	aa 156-168 of epsilon chain of Human CD3 protein (intracytoplasmic).
<b>Positive control</b>	WB: Jurkat whole cell lysate. Human, mouse and rat thymus tissue lysate. IHC-P: Pig and rat spleen tissue. Human tonsil tissue. Mouse epididymal fat pad tissue. Rat infarcted heart tissue. Flow Cytometry: Human peripheral blood lymphocytes. Jurkat cells. IHC-Fr: Mouse brain tissue.
<b>General notes</b>	This antibody is suitable for staining normal and neoplastic T cells in formalin-fixed, paraffin-embedded tissues.

### Properties

---

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.
<b>Storage buffer</b>	Preservative: 0.1% Sodium Azide Constituents: 1% BSA, Tris buffered saline, pH 7.5
<b>Purity</b>	Tissue culture supernatant
<b>Primary antibody notes</b>	This antibody is suitable for staining normal and neoplastic T cells in formalin-fixed, paraffin-embedded tissues.
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	SP7
<b>Isotype</b>	IgG

## Applications

Our [Abpromise guarantee](#) covers the use of **ab16669** in the following tested applications.

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

Application	Abreviews	Notes
Flow Cyt	★★★★☆	1/1000. <a href="#">ab172730</a> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. We recommend using Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ( <a href="#">ab150077</a> ) secondary antibody.
IHC-Fr	★★★★★	Use at an assay dependent concentration. PubMed: 18658050
IHC-P	★★★★★	1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Boil tissue section in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min.
WB	★★★★★	1/200. Predicted molecular weight: 23 kDa.
IHC-FoFr	★★★★★	Use at an assay dependent concentration.

## Target

### Function

The CD3 complex mediates signal transduction.

### Involvement in disease

Defects in CD3D are a cause of severe combined immunodeficiency autosomal recessive T-cell-negative/B-cell-positive/NK-cell-positive (T(-)/B(+)/NK(+)) SCID [MIM:608971]. A form of severe combined immunodeficiency (SCID), a genetically and clinically heterogeneous group of rare congenital disorders characterized by impairment of both humoral and cell-mediated immunity, leukopenia, and low or absent antibody levels. Patients present in infancy recurrent, persistent infections by opportunistic organisms. The common characteristic of all types of SCID is absence of T-cell-mediated cellular immunity due to a defect in T-cell development.

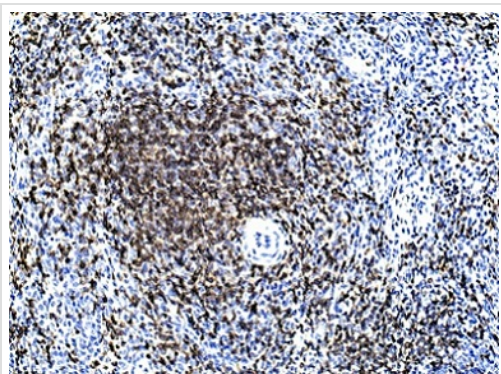
### Sequence similarities

Contains 1 ITAM domain.

### Cellular localization

Membrane.

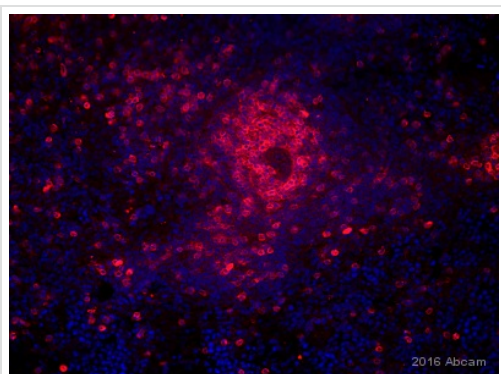
## Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] (ab16669)

This image is courtesy of an Abreview submitted by Carl Hobbs

Immunohistochemical analysis of Formaldehyde fixed, paraffin-embedded pig spleen tissue sections labelling CD3 with ab16669 at a dilution of 1/100. Biotin conjugated Goat Anti-Rabbit IgG was used as the secondary antibody. Antigen retrieval was heat mediated using citric acid.



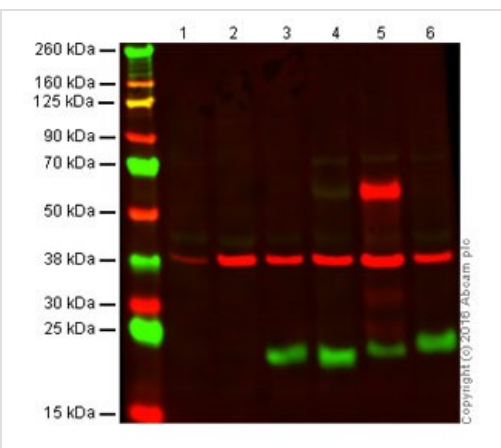
Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-CD3 antibody [SP7] (ab16669)

This image is courtesy of an Abreview submitted by Carl Hobbs

Immunohistochemical analysis of Formaldehyde fixed, frozen rat spleen tissue sections labelling CD3 with ab16669 at a dilution of 1/500. Biotin conjugated Goat Anti-Rabbit IgG at 1/300 dilution was used as the secondary antibody.

Cryostat sections (10 microns thick) of fresh frozen spleen were dried overnight. They were fixed in 10% Formalin in PBS pH7 for 15 minutes.

After secondary antibody incubation ( Goat anti-rabbit biotin ), streptavidin-Alexa 594 was applied.



Western blot - Anti-CD3 antibody [SP7] (ab16669)

**All lanes :** Anti-CD3 antibody [SP7] (ab16669) at 1/25 dilution

- Lane 1 :** THP1 whole cell lysate (-ve control)
- Lane 2 :** Raji whole cell lysate (-ve control)
- Lane 3 :** Jurkat whole cell lysate
- Lane 4 :** Human Thymus tissue lysate
- Lane 5 :** Mouse Thymus tissue lysate
- Lane 6 :** Rat Thymus tissue lysate

Lysates/proteins at 15 µg per lane.

### Secondary

**All lanes** : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution

Performed under reducing conditions.

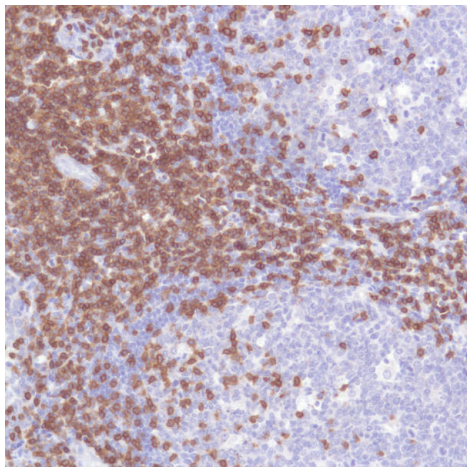
**Predicted band size:** 23 kDa

**Observed band size:** 23 kDa

Lanes 1 - 6: Merged signal (red and green).  
Green – [ab16669](#) observed at 23 kDa. Red - loading control, [ab8245](#), observed at 37 kDa.

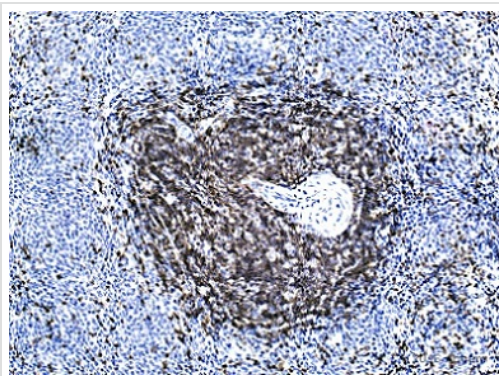
This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with [ab16669](#) and [ab8245](#) (loading control) overnight at 4°C. Antibody binding was detected using Goat Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed ([ab216776](#)) at a 1:10000 dilution for 1hr at room temperature and then imaged.

---



Immunohistochemical (Formalin / PFA fixed paraffin-embedded sections) staining of of Tonsil tissue labeling CD3 (SP7) with ab16669 at 1/150 dilution.

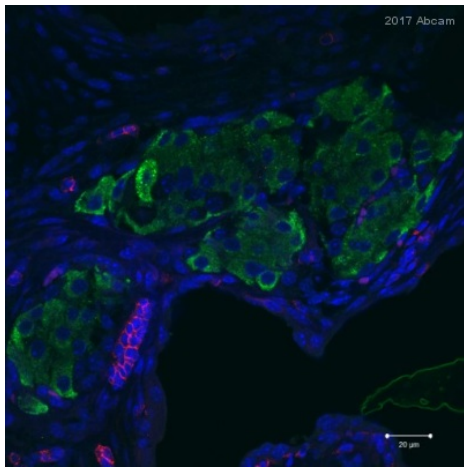
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] (ab16669)



Immunohistochemical analysis of Formaldehyde fixed, paraffin-embedded rat spleen tissue sections labelling CD3 with ab16669 at a dilution of 1/100. Biotin conjugated Goat Anti-Rabbit IgG at 1/300 dilution was used as the secondary antibody. Antigen retrieval was heat mediated using citric acid.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] (ab16669)

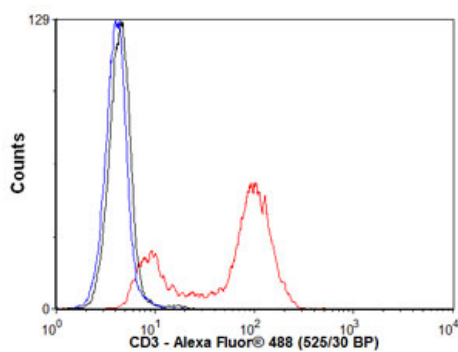
This image is courtesy of an Abreview submitted by Carl Hobbs



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] (ab16669)

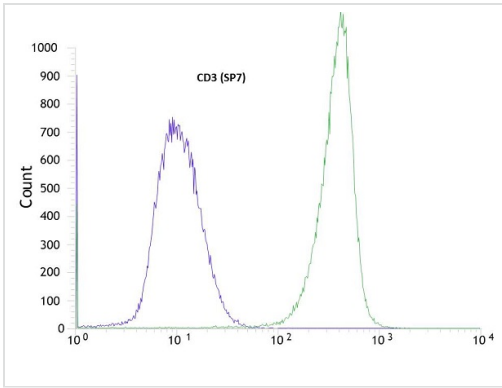
This image is courtesy of an Abreview submitted by Ying Li.

Ab16669 staining CD3 in Mouse Epididymal fat pad tissue sections by Immunohistochemistry (Formalin/PFA-fixed paraffin embedded sections). Tissue sections were fixed with formaldehyde, blocked with 5% serum for 4 hours at 25°C and permeabilized with Triton X-100. Samples were incubated with primary antibody (1/100 in PBST with BSA and goat serum) for 4°C at 12 hours. An Alexa Fluor® 568 goat anti-rabbit IgG (H + L) cross adsorbed was used as the secondary antibody.



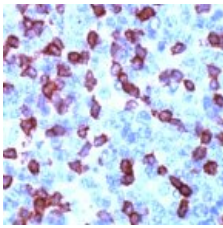
Flow Cytometry - Anti-CD3 antibody [SP7] (ab16669)

Human peripheral blood lymphocytes stained with ab16669 (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 (ab16669, 1/1000 dilution) for 30 min at 4°C. The secondary antibody used was [Goat Anti-Rabbit IgG H&L \(Alexa Fluor® 488\) \(ab150077\) secondary antibody](#) at 1/2000 dilution for 30 min at 4°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1μg/1x10<sup>6</sup> 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.



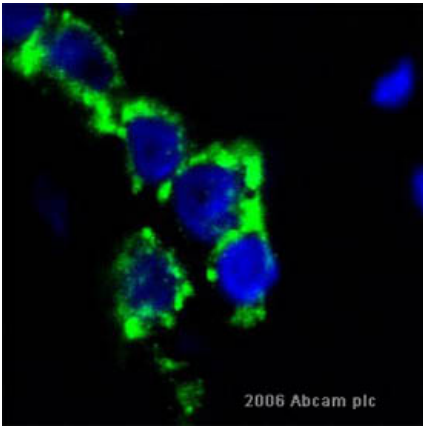
Flow cytometric analysis of rabbit anti-CD3 (SP7) antibody ab16669 (1/100) in Jurkats cells (green) compare to negative control of rabbit IgG (blue).

Flow Cytometry - Anti-CD3 antibody [SP7]  
(ab16669)



Human tonsil stained with ab16669.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7]  
(ab16669)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] (ab16669)

This image is courtesy of an Abreview submitted by Dr Mal Niladri

ab16669 staining rat infarcted heart tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

Myocardial infarction was produced in a rat model following the ligation of the left anterior descending (LAD) coronary artery. Tissue was harvested 6 w following infarct, fixed with Histochoice for 72 hr, paraffin sectioned and the slide was then baked prior to CD3 staining. ab16669 at 1/200 was incubated overnight at 4°C. The image was taken with a confocal laser scanning microscope and shows cells giving strong immunofluorescence staining for CD3 antigen (green), indicating presence of cells of T-lymphocytes origin in the infarct zone of the heart tissue, counterstained nuclei with DAPI (blue). Note, CD3 tended to be present in nests of 2-5 cells that were non-uniformly distributed in the infarct zone. In addition, the image shows that the CD3 localization is predominantly membrane based and to a certain extent intracytoplasmic.



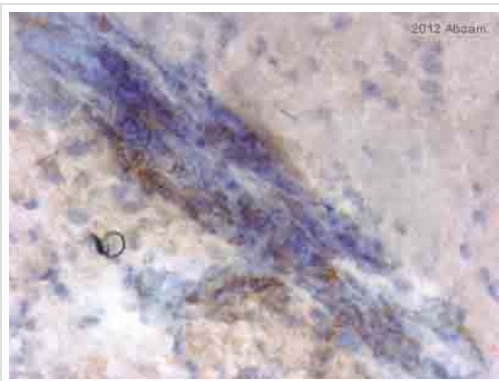
Western blot - Anti-CD3 antibody [SP7] (ab16669)

Anti-CD3 antibody [SP7] (ab16669) at 1/25 dilution + Jurkat cell lysate

**Predicted band size:** 23 kDa

**Observed band size:** 19 kDa

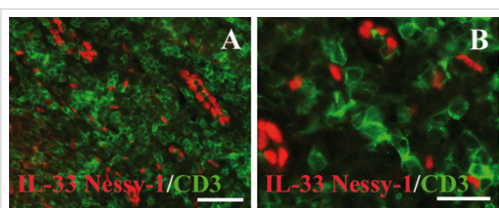




Immunohistochemistry (Frozen sections) - Anti-CD3 antibody [SP7] (ab16669)

Image courtesy of an anonymous Abreview.

ab16669 staining CD3 in murine brain tissue by Immunohistochemistry (Frozen sections). Tissue was fixed in acetone, blocked using 5% serum for 30 minutes at 25°C and then incubated with ab16669 at a 1/200 dilution for 18 hours at 4°C. The secondary used was an undiluted HRP conjugated goat anti-rabbit polyclonal.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] (ab16669)

Image from Mbousson C et al., PLoS One. 2008 Oct 6;3(10):e33331. Fig 2.; doi:10.1371/journal.pone.0003331; October 6, 2008, PLoS ONE 3(10): e33331.

Immunohistochemical analysis of Human tonsil tissue, staining CD3 (green) with ab16669.

Antigen retrieval was performed by heat mediation in citrate buffer (pH 6) and blocked with 5% goat serum and 5% BSA for 1 hour at room temperature. Samples were incubated with primary antibody (1/100) overnight at 4°C. A Cy3®-conjugated anti-rabbit IgG was used as the secondary antibody.

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

## Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <http://www.abcam.com/abpromise> or contact our technical team.

## Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors