


Product datasheet

Anti-CD3 antibody [SP7] ab16669

RabMAb

★★★★☆ 73 Abreviews 449 References 12 Images

Overview

Product name	Anti-CD3 antibody [SP7]
Description	Rabbit monoclonal [SP7] to CD3
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt, IHC-P, WB, mIHC
Species reactivity	Reacts with: Mouse, Rat, Human Predicted to work with: Sheep, Rabbit, Horse, Chicken, Cow, Cat, Dog, Pig, Baboon, Woodchuck 
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Recombinant Human CD3 epsilon protein (ab114153), Jurkat whole cell lysate. Human, mouse and rat thymus tissue lysate. IHC-P: Rat spleen tissue. Human tonsil tissue. Mouse epididymal fat pad and lymph node tissues. Rat infarcted heart and spleen tissues. Flow Cyt: Human peripheral blood lymphocytes. Jurkat cells.
General notes	<p>We recommend ab135372 as an alternative.</p> <p>This antibody is suitable for staining normal and neoplastic T cells in formalin-fixed, paraffin-embedded tissues.</p> <p>Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).</p> <p>See other anti-rabbit secondary antibodies that can be used with this antibody.</p> <p>This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.</p> <p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.50 Preservative: 0.1% Sodium azide Constituents: Tissue culture supernatant, Tris buffered saline, 1% BSA
Purity	Tissue culture supernatant
Primary antibody notes	This antibody is suitable for staining normal and neoplastic T cells in formalin-fixed, paraffin-embedded tissues.
Clonality	Monoclonal
Clone number	SP7
Isotype	IgG

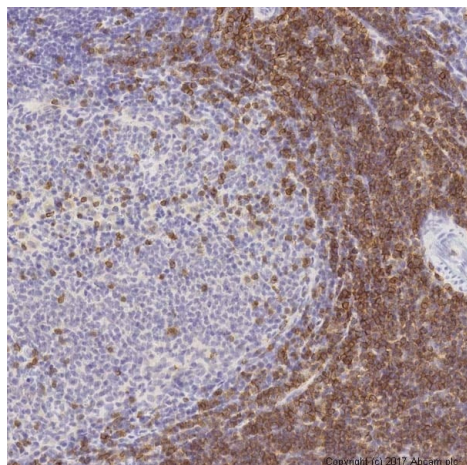
Applications

The Abpromise guarantee 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	★★★★★ (2)	1/1000. ab172730 - 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) (ab150077) secondary antibody.
IHC-P	★★★★★ (52)	1/150. 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)	1/25. Predicted molecular weight: 23 kDa.
mIHC		Use at an assay dependent concentration.

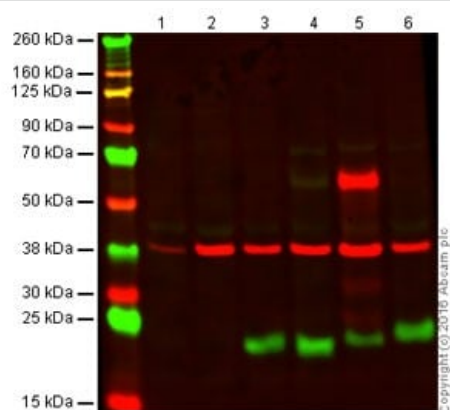
Target

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.



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

IHC image of CD3 staining in a formalin fixed, paraffin embedded normal rat spleen tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab16669 at 1/100 dilution for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



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

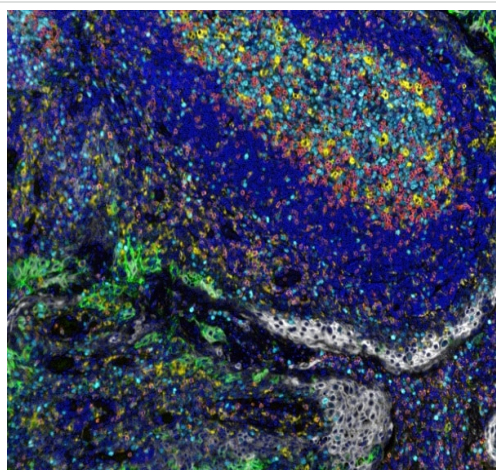
Performed under reducing conditions.

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.



Multiplex immunohistochemistry - Anti-CD3 antibody [SP7] (ab16669)

Fluorescence multiplex immunohistochemical analysis of normal human tonsil tissue (formalin-fixed paraffin-embedded section).

Merged staining of anti-PD1 ([ab237728](#); orange; Opal™520), anti-PDL1 ([ab237726](#); green; Opal™540), anti-CD68 ([ab192847](#); yellow; Opal™570), anti-CD3 (ab16669; red; Opal™620), anti-Ki67 ([ab16667](#); light blue; Opal™650) and anti-PanCK ([ab7753](#); grey; Opal™690).

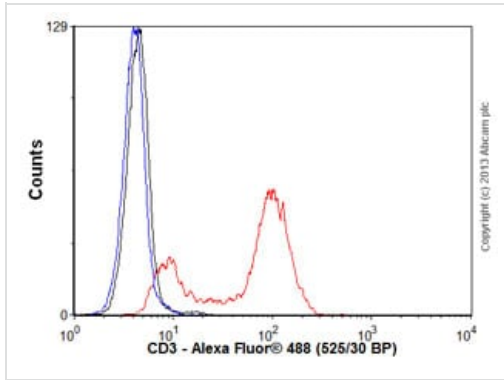
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 7-color automation IHC kit (NEL821001KT, Akoya Biosciences®).

The section was incubated in six rounds of staining; in the order of [ab237728](#) (1/500 dilution), [ab237726](#) (1/500 dilution), [ab192847](#) (1/300 dilution), ab16669 (1/300 dilution), [ab16667](#) (1/200 dilution) and [ab7753](#) (1/200 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (Leica ER1, pH6.0, 30 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

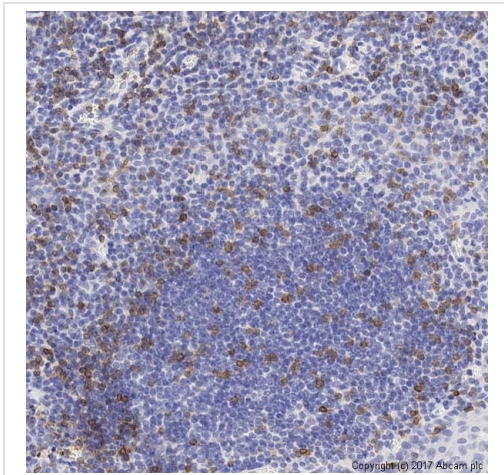
DAPI (dark blue) was used as a nuclear counter stain.

Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra Polaris.



Flow Cytometry - Anti-CD3 antibody [SP7]
(ab166669)

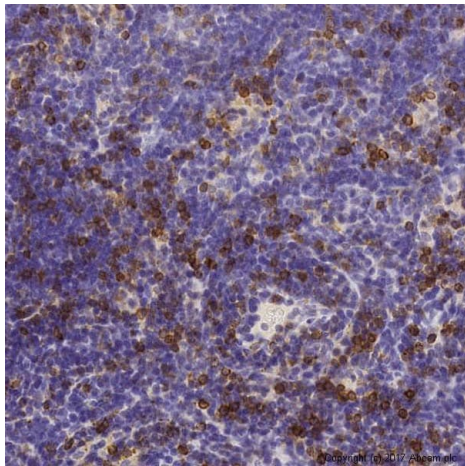
Human peripheral blood lymphocytes stained with ab16669 (red line). Human whole blood was processed using a modified protocol based on Chow *et al*, 2005 (PMID: 16080188). In brief, human whole blood was fixed in 4% formaldehyde (methanol-free) for 10 min at 22°C. Red blood cells were then lysed by the 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/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-CD3 antibody [SP7]
(ab166669)

IHC image of CD3 staining in a formalin fixed, paraffin embedded normal human tonsil tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab16669 at 1/100 dilution for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

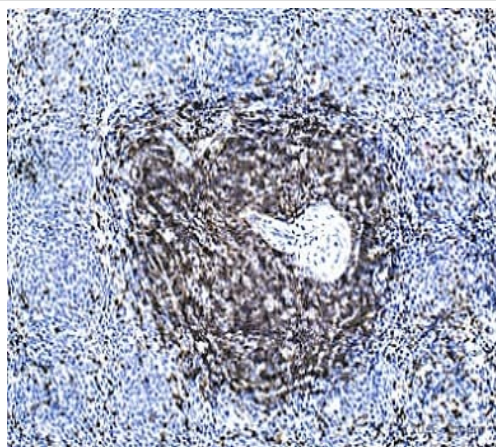
*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



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

IHC image of CD3 staining in mouse lymph node formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol B. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab16669 at 1/100 dilution for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

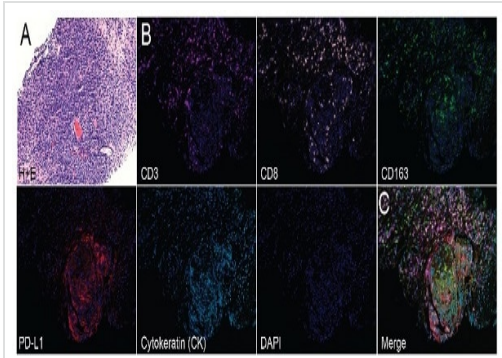
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times



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

This image is courtesy of an Abreview submitted by Carl Hobbs

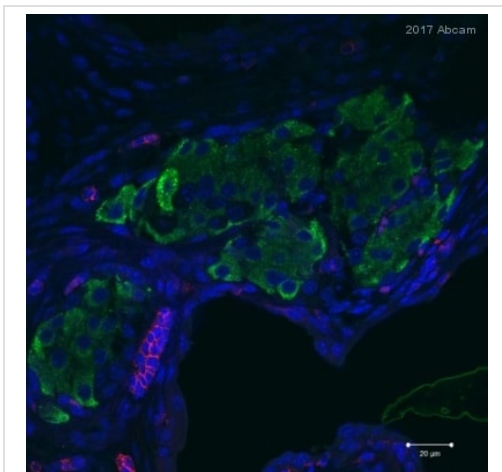
Immunohistochemical analysis of Formaldehyde fixed, paraffin-embedded rat spleen tissue sections labelling CD3 with ab16669 at a dilution of 1/100. Biotin conjugated Goat Anti-Rabbit IgG at 1/300 dilution was used as the secondary antibody. Antigen retrieval was heat mediated using citric acid.



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

Image from Graff JN et al., *Oncotarget* 7(33), 52810 - 52817. Fig 2.; doi: 10.18632/oncotarget.10547. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/3.0/>.

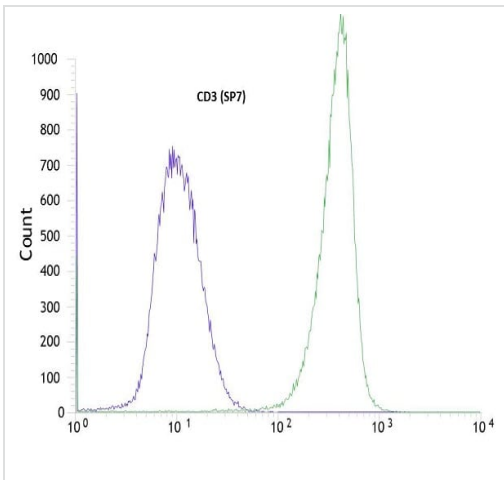
IHC using multi-spectral imaging on human lymph node (A-C) obtained from men with mCRPC. A) H+E staining and B) single-color images (plus nuclear stain; DAPI) of CD3 (ab16669), CD8 (ab101500), CD163, PD-L1, cytokeatin (CK), DAPI and C) merged. H+E staining at 20X magnification; multi-spectral images 200X magnification.



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

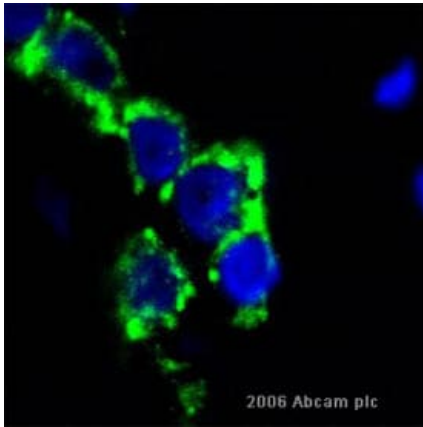
This image is courtesy of an Abreview submitted by Ying Li.

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)

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

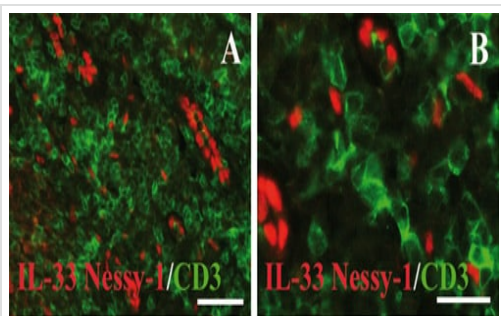


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7]
(ab16669)

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.



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.

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

Image from Mboussion 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.

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