abcam

Product datasheet

Anti-CD3 antibody [SP7] ab16669

RabMAb

63 Abreviews 165 References 14 Images

Overview

Product name	Anti-CD3 antibody [SP7]
Description	Rabbit monoclonal [SP7] to CD3
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt, IHC-Fr, IHC-P, WB, IHC-FoFr
Species reactivity	**Reacts with:** Mouse, Rat, Human, Pig **Predicted to work with:** Sheep, Rabbit, Horse, Chicken, Cow, Cat, Dog, Baboon, Woodchuck
Immunogen	Synthetic peptide within Human CD3 aa 150 to the C-terminus. The exact sequence is proprietary. Database link: P07766
Positive control	WB: Recombinant Human CD3 epsilon protein (ab114153), 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.
General notes	We recommend ab135372 as an alternative. This antibody is suitable for staining normal and neoplastic T cells in formalin-fixed, paraffin-embedded tissues. Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077). See other anti-rabbit secondary antibodies that can be used with this antibody.

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

Our Abpromise guarantee covers the use of **ab16669** in the following tested applications.

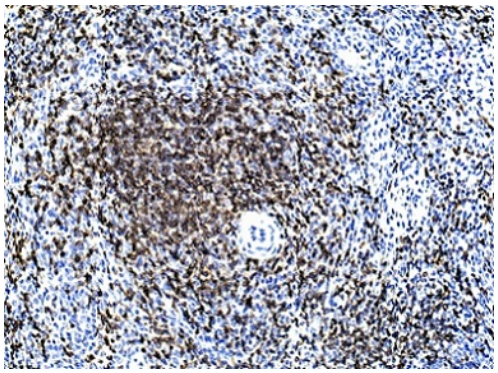
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt	★★★★☆	1/1000. 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-Fr	★★★★★	Use at an assay dependent concentration. PubMed: 18658050
IHC-P	★★★★★	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	★★★★★	1/200. 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.

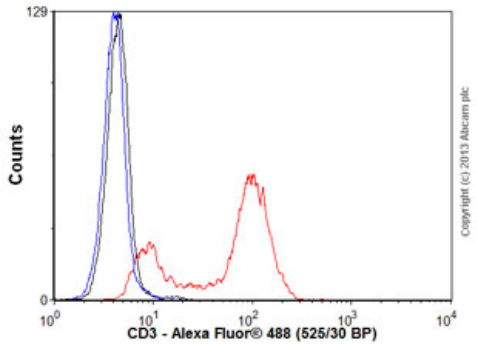
Images



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

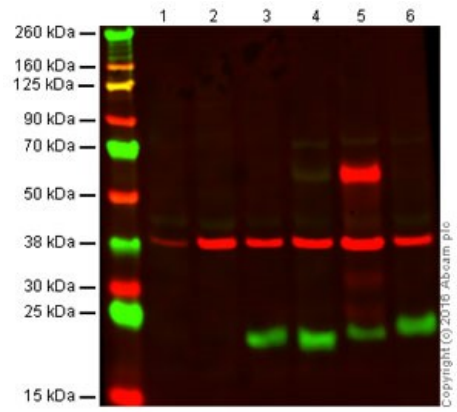
This image is courtesy of an Abreview submitted by Carl Hobbs

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.



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 lyzed 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/1x10^6 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.



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

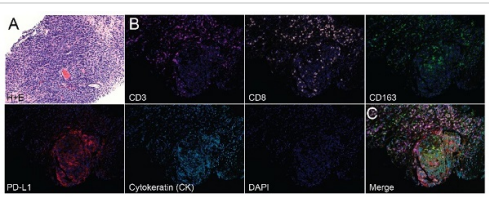
Performed under reducing conditions.

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

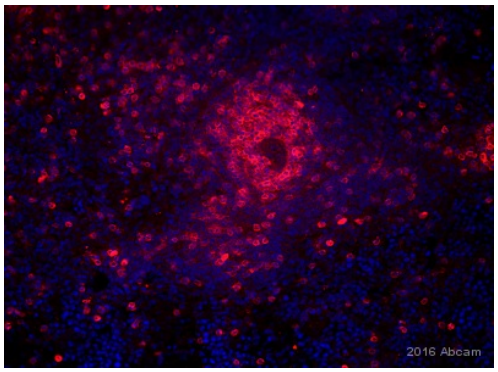
temperature and then imaged.



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.

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, cytokeratin (CK), DAPI and C) merged. H+E stating at 20X magnification; multi-spectral images 200X magnification.

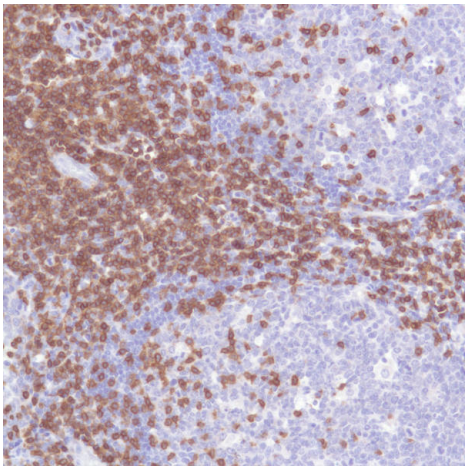


Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-CD3 antibody [SP7] (ab16669)

This image is courtesy of an Abreview submitted by Carl Hobbs

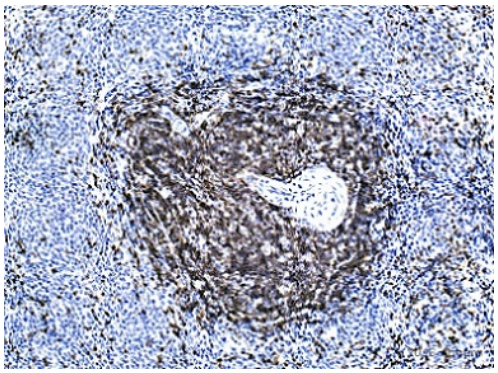
Immunohistochemical analysis of Formaldehyde fixed, frozen rat spleen tissue sections labelling CD3 with ab16669 at a dilution of 1/500. Biotin conjugated Goat Anti-Rabbit IgG at 1/300 dilution was used as the secondary antibody.

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.



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

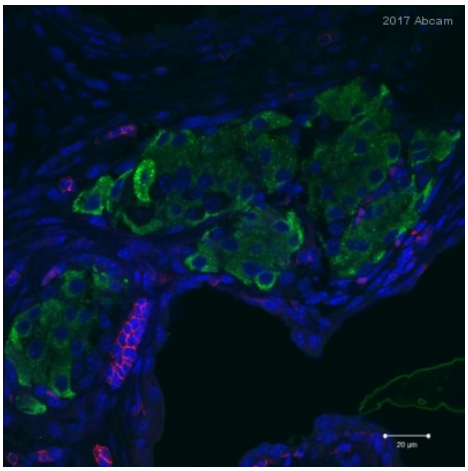
Immunohistochemical (Formalin / PFA fixed paraffin-embedded sections) staining of of Tonsil tissue labeling CD3 (SP7) with ab16669 at 1/150 dilution.



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

This image is courtesy of an Abreview submitted by Carl Hobbs

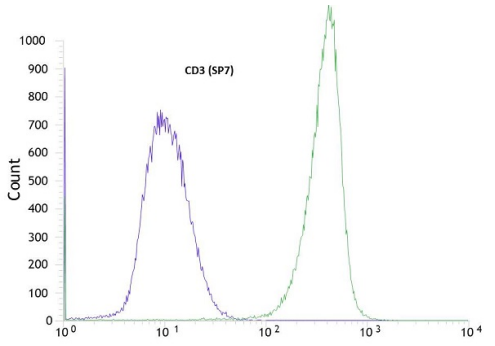
Immunohistochemical analysis of Formaldehyde fixed, paraffin-embedded rat spleen tissue sections labelling CD3 with ab16669 at a dilution of 1/100. Biotin conjugated Goat Anti-Rabbit IgG at 1/300 dilution was used as the secondary antibody. Antigen retrieval was heat mediated using citric acid.



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

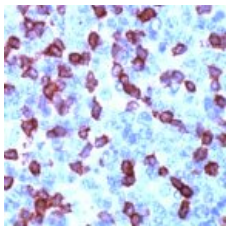
This image is courtesy of an Abreview submitted by Ying Li.

Ab16669 staining CD3 in Mouse Epidydimal 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.



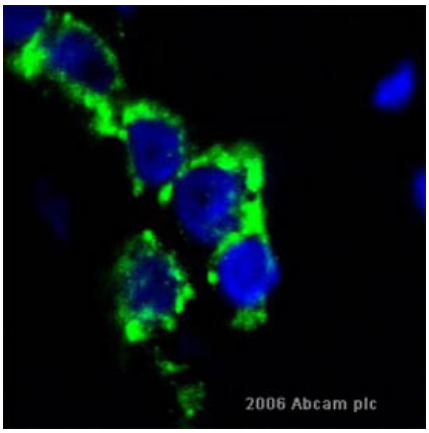
Flow Cytometry - Anti-CD3 antibody [SP7] (ab16669)

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)

Human tonsil stained with ab16669.

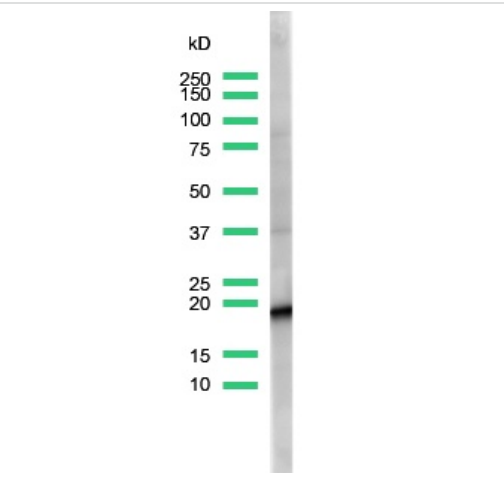


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] (ab16669)

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).

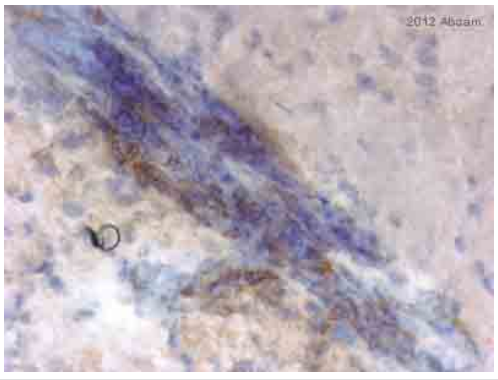
Myocardial infarction was produced in a rat model following the ligation of the left anterior descending (LAD) coronary artery. Tissue was harvested 6 w following infarct, fixed with Histochoice for 72 hr, paraffin sectioned and the slide was then baked prior to CD3 staining. ab16669 at 1/200 was incubated overnight at 4°C. The image was taken with a confocal laser scanning microscope and shows cells giving strong inmmunofluorescence 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

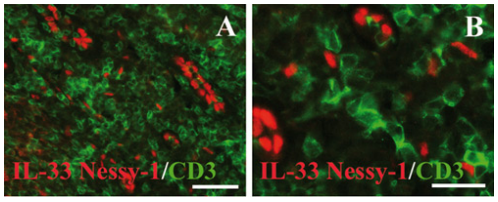
Predicted band size: 23 kDa
Observed band size: 19 kDa
why is the actual band size different from the predicted?



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 Mbussion C et al., PLoS One. 2008 Oct 6;3(10):e3331. Fig 2.; doi:10.1371/journal.pone.0003331; October 6, 2008, PLoS ONE 3(10): e3331.

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.

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