abcam

Product datasheet

Anti-CD3 antibody [SP7] - BSA and Azide free ab205228

RabMAb

★★★★★ 1 Abreviews 11 References 21 Images

Overview

Product name	Anti-CD3 antibody [SP7] - BSA and Azide free
Description	Rabbit monoclonal [SP7] to CD3 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, 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: Human tonsil tissue. Mouse epididymal fat pad and lymph node tissues. Rat infarcted heart and spleen tissues. Flow Cyt (intra): Human peripheral blood lymphocytes. Jurkat cells. mIHC: Human tonsil tissue, human duodenum tissue, human colon tissue. Mouse and rat spleen tissue.
General notes	This is the BSA and azide free formulation of **ab16669**. This antibody is suitable for staining normal and neoplastic T cells in formalin-fixed, paraffin-embedded tissues. **This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.** 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. 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

Properties

Form	Liquid

Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at +4°C. Do Not Freeze.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	SP7
Isotype	IgG

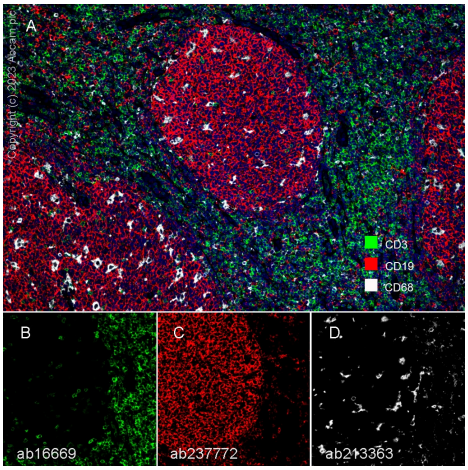
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab205228 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 (Intra)		Use at an assay dependent concentration. **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.
WB		Use at an assay dependent concentration. Predicted molecular weight: 23 kDa.
IHC-P	★★★★★ (1)	Use at an assay dependent concentration. 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.
mIHC		Use at an assay dependent concentration. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2)

Images



Multiplex immunohistochemistry - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

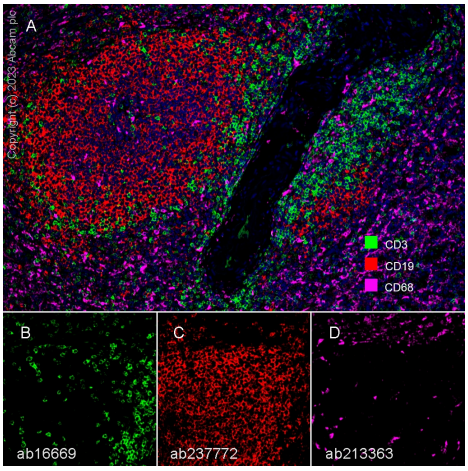
This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

Panel A: merged staining of anti-CD68 (gray; Opal™690), anti-CD3 (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human tonsil. Secondary antibody was Opal Polymer HRP Ms + Rb, and counterstaining was with DAPI.

Panel B: anti-CD3 stained on T cells with **ab16669** at 1/500 dilution
Panel C: anti-CD19 stained on B cells with **ab237772** at 1/5000 dilution
Panel D: anti-CD68 stained on macrophages with **ab213363** 1/500 dilution

The section was incubated in three rounds of staining: in the order of **ab213363** and **ab16669** for 30 mins, then **ab237772** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

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.



Multiplex immunohistochemistry - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

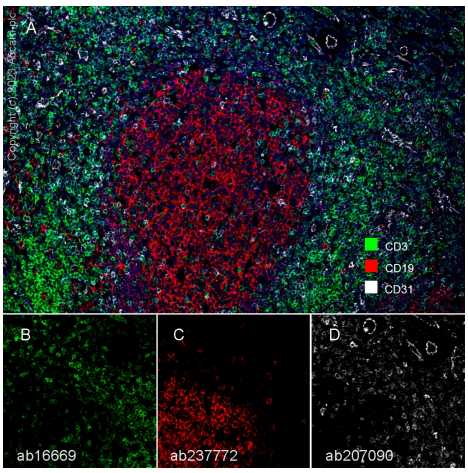
This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

Panel A: merged staining of anti-CD68 (magenta; Opal™690), anti-CD3 (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human spleen. Secondary antibody was Opal Polymer HRP Ms + Rb, and counterstaining was with DAPI.

Panel B: anti-CD3 stained on T cells with **ab16669** at 1/500 dilution
Panel C: anti-CD19 stained on B cells with **ab237772** at 1/5000 dilution
Panel D: anti-CD68 stained on macrophages with **ab213363** 1/500 dilution

The section was incubated in three rounds of staining: in the order of **ab213363** and **ab16669** for 30 mins, then **ab237772** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

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.

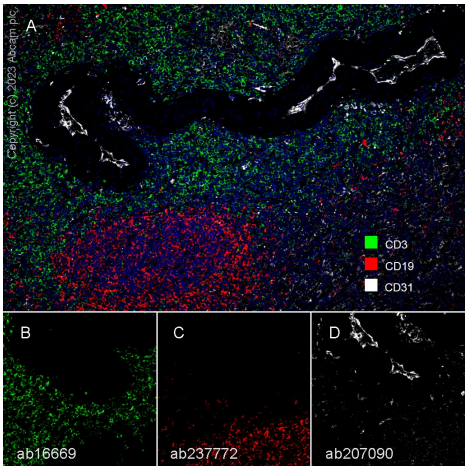


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

Panel A: merged staining of anti-CD31 (gray; Opal™690), anti-CD3 (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human tonsil. Secondary antibody was Opal Polymer HRP Ms + Rb, nuclear counterstain was DAPI.
Panel B: anti-CD3 stained on T cells with **ab16669** at 1/500 dilution
Panel C: anti-CD19 stained on B cells with **ab237772** at 1/5000 dilution
Panel D: anti-CD31 stained on endothelial cells and immune cell subsets with **ab207090** at 1/500 dilution
The section was incubated in three rounds of staining: in the order of **ab207090** and **ab16669** for 30 mins, then **ab237772** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.
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. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope

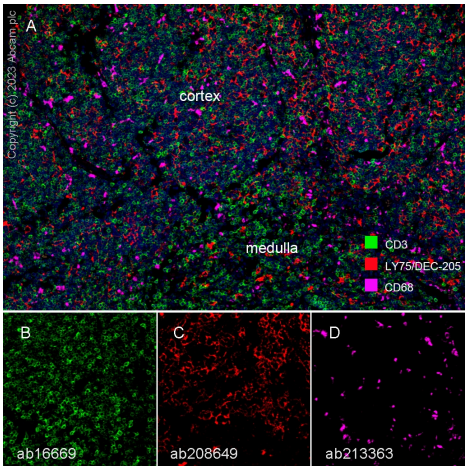
retrieval solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

Panel A: merged staining of anti-CD31 (gray; Opal™690), anti-CD3 (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human spleen. Secondary antibody was Opal Polymer HRP Ms + Rb, nuclear counterstain was DAPI.
Panel B: anti-CD3 stained on T cells with **ab16669** at 1/500 dilution
Panel C: anti-CD19 stained on B cells with **ab237772** at 1/5000 dilution
Panel D: anti-CD31 stained on endothelial cells with **ab207090** at 1/500 dilution
The section was incubated in three rounds of staining: in the order of **ab207090** and **ab16669** for 30 mins, then **ab237772** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.
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. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Multiplex immunohistochemistry - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Human thymus tissue labeling CD3 with **ab16669** at 1/500 dilution, LY75/DEC-205 with **ab208649** at 1/15000, and CD68 with **ab213363** at 1/500 dilution.

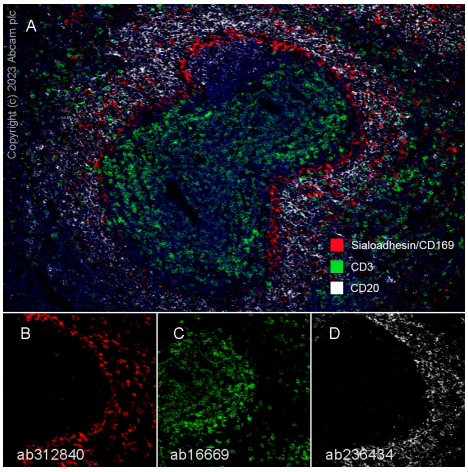
Panel A: merged staining of anti-CD68 (magenta; Opal™690), anti-CD3 (green; Opal™520) and anti-LY75/DEC-205 (red; Opal™570) on human thymus.
Panel B: anti-CD3 stained on T cells.
Panel C: anti-LY75/DEC-205 stained on thymic cortical epithelium and dendritic cells.
Panel D: anti-CD68 stained on macrophages.

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 **ab213363**, **ab16669**, and

ab208649 for 30 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.



Multiplex immunohistochemistry - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

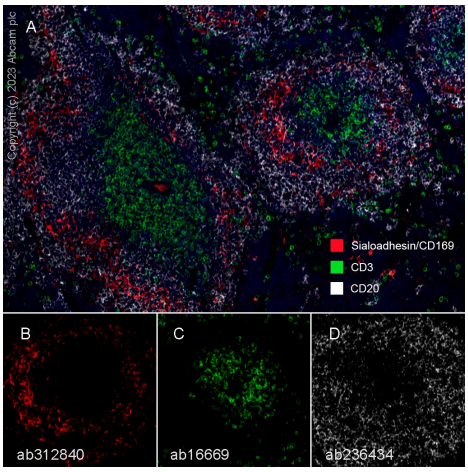
This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Rat spleen tissue labeling Sialoadhesin/CD169, CD3 and CD20 with **ab312840** at 1/100 dilution, **ab16669** at 1:150 dilution and **ab236434** at 1:5000 dilution.

Panel A: merged staining of anti-Sialoadhesin/CD169 (red; Opal™690), anti-CD3 (green; Opal™520) and anti-CD20 (gray; Opal™570) on rat spleen.
Panel B: anti-Sialoadhesin/CD169 stained on macrophages.
Panel C: anti-CD3 stained on T cells.
Panel D: anti-CD20 stained on B cells.

The section was incubated in three rounds of staining: in the order of **ab312840**, **ab16669**, and **ab236434** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

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.



Multiplex immunohistochemistry - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

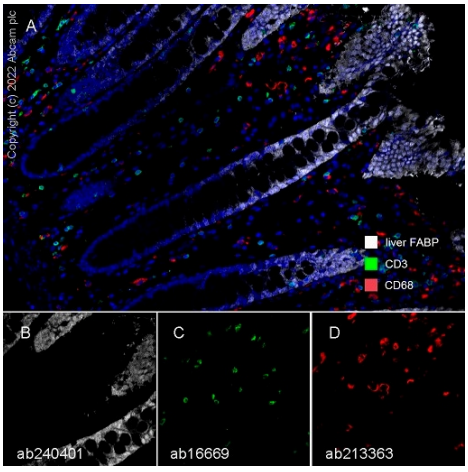
This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Mouse spleen tissue labeling Sialoadhesin/CD169, CD3 and CD20 with **ab312840** at 1/100 dilution, **ab16669** at 1:150 dilution and **ab236434** at 1:5000 dilution.

Panel A: merged staining of anti-Sialoadhesin/CD169 (red; Opal™690), anti-CD3 (green; Opal™520) and anti-CD20 (gray; Opal™570) on mouse spleen.
Panel B: anti-Sialoadhesin/CD169 stained on macrophages.
Panel C: anti-CD3 stained on T cells.
Panel D: anti-CD20 stained on B cells.

The section was incubated in three rounds of staining: in the order of **ab312840**, **ab16669**, and **ab236434** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

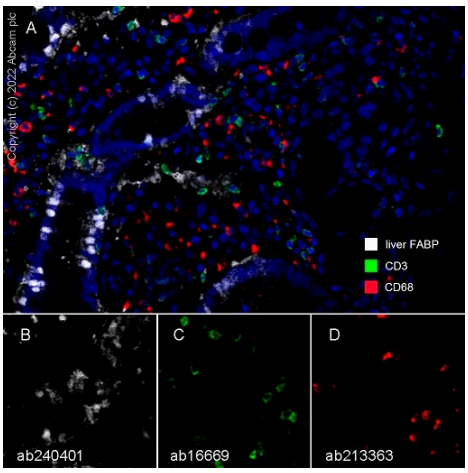
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

Fluorescence multiplex immunohistochemical analysis of the human colon (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-liver FABP (**ab240401**, gray; Opal™690), anti-CD3 (**ab16669**, green; Opal™520) and anti-CD68 (**ab213363**, red; Opal™570) on human colon. Panel B: anti-liver FABP stained on enterocytes. Panel C: anti-CD3 stained on T cells. Panel D: anti-CD68 stained on macrophages. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of **ab240401** (1/8000 dilution), **ab16669** (1/150 dilution), and **ab213363** (1/500 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

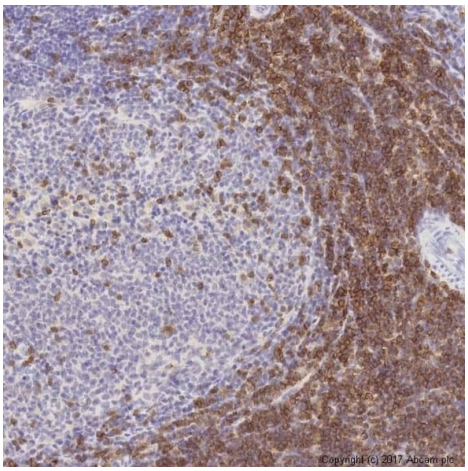
This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.



Multiplex immunohistochemistry - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

Fluorescence multiplex immunohistochemical analysis of the human duodenum (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-liver FABP (**ab240401**, gray; Opal™690), anti-CD3 (**ab16669**, green; Opal™520) and anti-CD68 (**ab213363**, red; Opal™570) on human duodenum. Panel B: anti-liver FABP stained on enterocytes. Panel C: anti-CD3 stained on T cells. Panel D: anti-CD68 stained on macrophages. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of **ab240401** (1/8000 dilution), **ab16669** (1/150 dilution), and **ab213363** (1/500 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

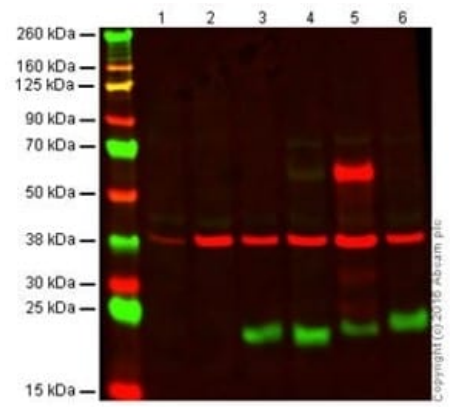


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab205228)

All lanes : Anti-CD3 epsilon 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

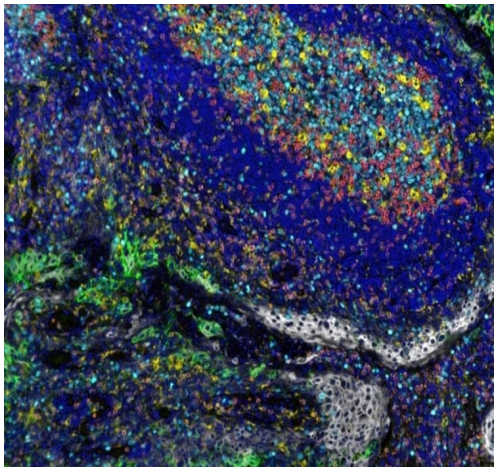
Developed using the ECL technique.

Predicted band size: 23 kDa
Observed band size: 23 kDa

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab205228)

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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).

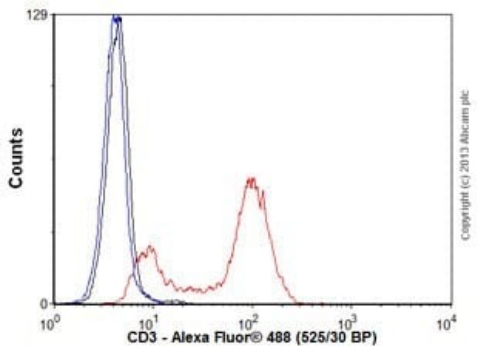
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 7-color automation IHC kit (NEL821001KT, Akoya Biosciences®).

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.

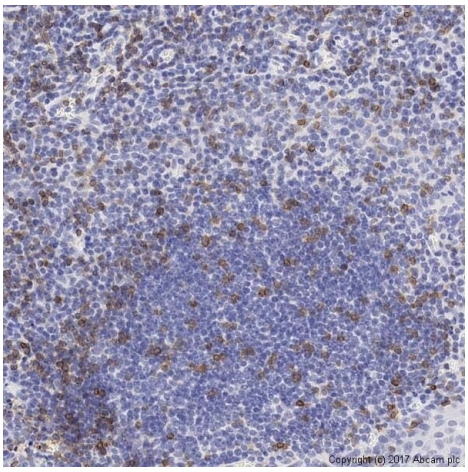


Flow Cytometry (Intracellular) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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.



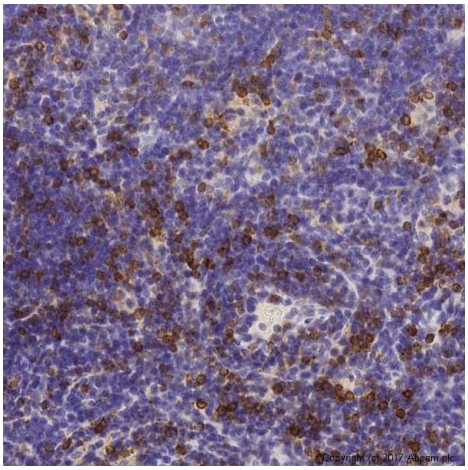
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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.

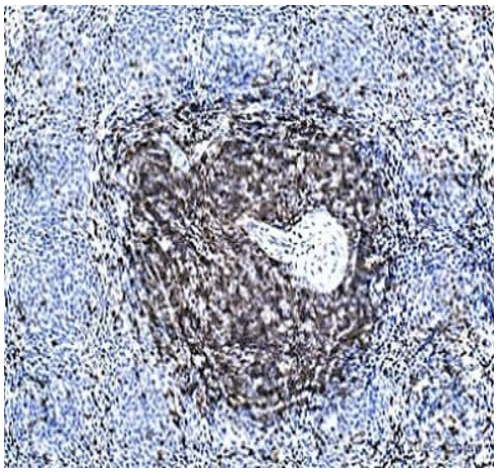


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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.

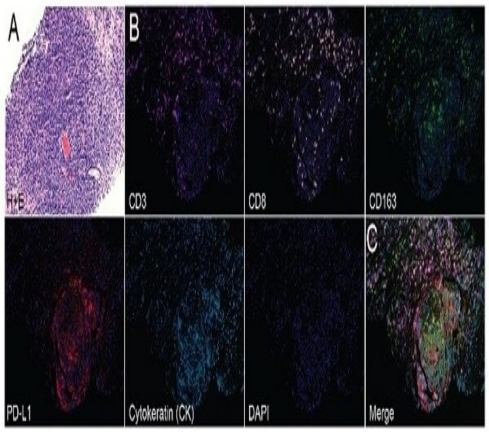


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

This image is courtesy of an Abreview submitted by Carl Hobbs

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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.

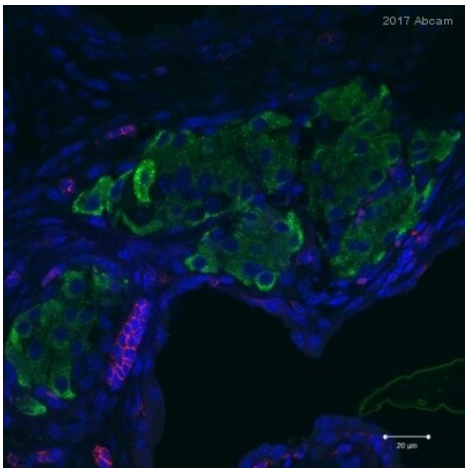


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

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/.

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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.

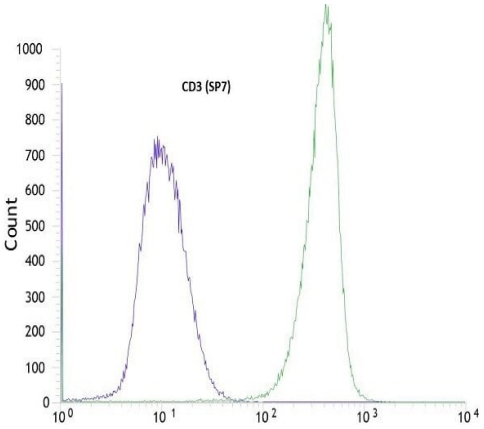


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

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

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

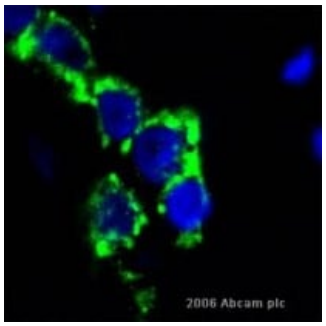
ab16669 staining CD3 in Mouse Epididymal fat pad tissue sections by Immunohistochemistry (Formalin/PFA-fixed paraffin embedded sections). Tissue sections were fixed with formaldehyde, blocked with 5% serum for 4 hours at 25°C and permeabilized with Triton X-100. Samples were incubated with primary antibody (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 (Intracellular) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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



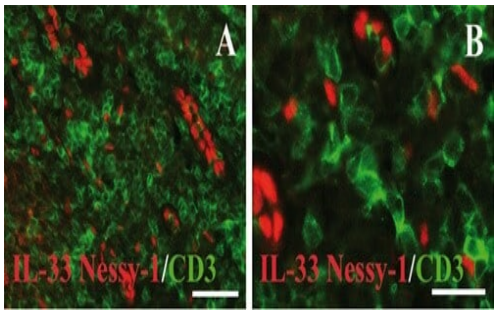
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

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

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7] - BSA and Azide free (ab205228)

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.

This data was developed using **ab16669**, the same antibody clone in a different buffer formulation.

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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