abcam

ab205228

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

Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228) is a rabbit monoclonal antibody provided in a PBS only buffer for easy conjugation detecting CD3 epsilon in **Western Blot, Flow Cytometry (Intra), IHC-P, mIHC**. Suitable for **Human, Mouse, Rat**.

- BSA, sodium azide, and glycerol-free for easy conjugation
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Over 10 publications

RabMAb | Advanced Validation

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	SP7
Purification technique	Affinity purification Protein A
Concentration	1.001 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	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.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Cow, Woodchuck, Rabbit, Baboon, Chicken, Horse, Pig, Dog, Cat
Dilution info	-
Notes	-

WB

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Predicted

Species	Cow, Woodchuck, Rabbit, Baboon, Chicken, Horse, Pig, Dog, Cat
Dilution info	-
Notes	-

mIHC

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Predicted

Species	Cow, Woodchuck, Rabbit, Baboon, Chicken, Horse, Pig, Dog, Cat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	-
Notes	Boil tissue section in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min.

Species	Rat
Dilution info	-
Notes	Boil tissue section in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min.

Species	Human
Dilution info	-
Notes	Boil tissue section in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Predicted

Species	Cow, Woodchuck, Rabbit, Baboon, Chicken, Horse, Pig, Dog, Cat
Dilution info	-

Notes	-

Target data

See full target information CD3E

Function	Part of the TCR-CD3 complex present on T-lymphocyte cell surface that plays an essential role in adaptive immune response (PubMed:15294938, PubMed:15546002, PubMed:2470098, PubMed:40592325, PubMed:8490660). When antigen presenting cells (APCs) activate T-cell receptor (TCR), TCR-mediated signals are transmitted across the cell membrane by the CD3 chains CD3D, CD3E, CD3G and CD247/CD3Z (PubMed:2470098, PubMed:40592325). All CD3 chains contain immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic domain (PubMed:2470098, PubMed:40592325). Upon TCR engagement, these motifs become phosphorylated by Src family protein tyrosine kinases LCK and FYN, resulting in the activation of downstream signaling pathways (PubMed:2470098, PubMed:40592325). CD3E ITAM phosphorylation creates docking sites for the protein kinase ZAP70 leading to ZAP70 phosphorylation and its conversion into a catalytically active enzyme (By similarity). In addition of this role of signal transduction in T-cell activation, CD3E plays an essential role in correct T-cell development (By similarity). Also participates in internalization and cell surface down-regulation of TCR-CD3 complexes via endocytosis sequences present in CD3E cytosolic region (PubMed:10384095, PubMed:26507128). In addition to its role as a TCR coreceptor, it serves as a receptor for ITPRIPL1 (PubMed:38614099). Ligand recognition inhibits T-cell activation by promoting interaction with NCK1, which prevents CD3E-ZAP70 interaction and blocks the ERK-NFkB signaling cascade and calcium influx (PubMed:12110186, PubMed:38614099).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Aliquoting information	Upon delivery aliquot
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228) is a rabbit monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunohistochemistry (IHC-P), Multiplex IHC (mIHC) in Human, Mouse, Rat samples.

What is the molecular weight of CD3 epsilon?
Anti-CD3 epsilon [SP7] - BSA and Azide free (ab205228) specifically detects a band for CD3 epsilon (UniProt: P07766) at a molecular weight of 23kDa.

Collaboration
Anti-CD3 epsilon [SP7] - BSA and Azide free (ab205228) 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-CD3 epsilon [SP7] - BSA and Azide free (ab205228) was first used in a scientific publication in 2015 and has been cited over 10 times in peer-reviewed journals.

Other related products
We have a range of other formats of antibody clone [SP7] also available for your convenience: ab16669, ab21703, Carrier free - ab205228, ab238627, ab279474

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

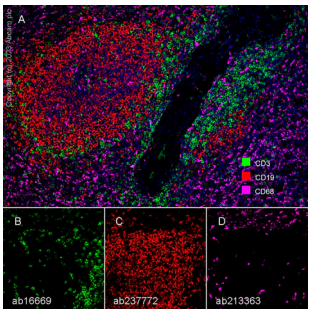
We do not recommend this combination. It is not covered by our product promise.

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.

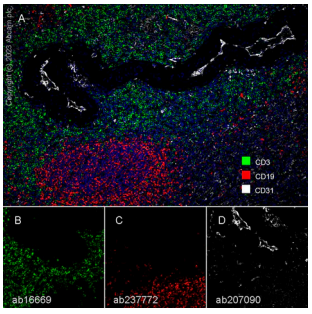
27 product images



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

CD3 epsilon Multiplex immunohistochemistry staining using rabbit Anti-CD3 epsilon antibody

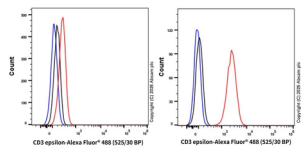
This data was developed using ab16669, the same antibody clone in a different buffer formulation. Panel A: merged staining of anti-CD68 (magenta; Opal™690), anti-CD3 epsilon (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human spleen. Secondary antibody was Opal Polymer HRP Ms + Rb, and couterstaining was with DAPI. Panel B: anti-CD3 epsilon 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 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Multiplex immunohistochemistry staining using rabbit Anti-CD3 epsilon antibody

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 epsilon (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 epsilon 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



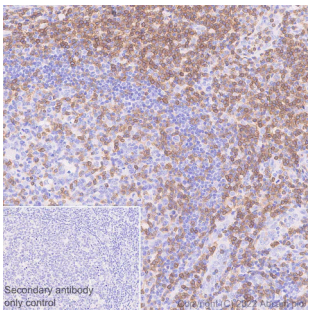
Flow Cytometry (Intracellular) - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Flow Cytometry (Intracellular) staining using rabbit Anti-CD3 epsilon antibody

This data was developed using ab16669, the same antibody clone in a different buffer formulation. Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Left: U-937 (Human histiocytic lymphoma monocyte) / Right: Jurkat (Human T cell leukemia T lymphocyte) cells labelling CD3 epsilon with ab16669 at 1/100 dilution / Red compared with an isotype control Rabbit monoclonal IgG (ab172730) / Black and unlabelled control cell without incubation with primary antibody and secondary antibody / Blue.

Goat anti-Rabbit IgG H&L (Alexa Fluor® 488, ab150081) preadsorbed at 1/5000 dilution was used as the secondary antibody.

Negative control: U-937 (PMID: 40579234)



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

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human tonsil tissue using rabbit Anti-CD3 epsilon antibody

This data was developed using ab16669, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD3 epsilon with ab16669 at 1/150 dilution.

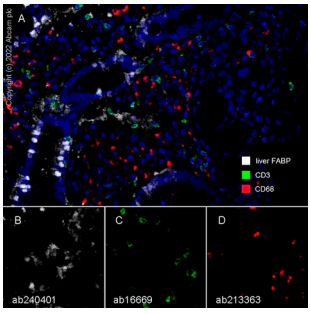
Positive staining on human tonsil

The primary antibody was incubated for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Heat mediated antigen retrieval was performed with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins.

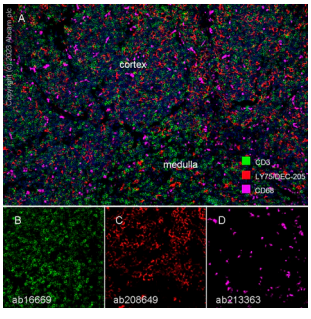


Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Multiplex immunohistochemistry staining of Human duodenum tissue using rabbit Anti-CD3 epsilon antibody

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 epsilon (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 epsilon 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.

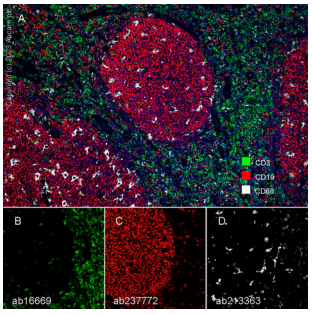


Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Multiplex immunohistochemistry staining of Human thymus using rabbit Anti-CD3 epsilon antibody

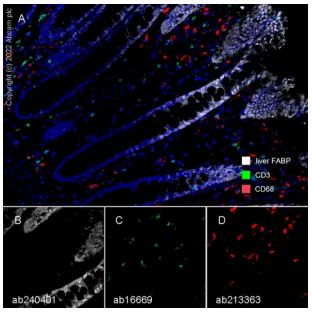
This data was developed using ab16669, the same antibody clone in a different buffer formulation. Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Human thymus tissue labeling CD3 epsilon with ab16669 at 1/500 dilution, LY75/DEC-205 with ab208649 at

1/15000, and CD68 with ab213363 at 1/500 dilution. Panel A: merged staining of anti-CD68 (magenta; Opal™690), anti-CD3 epsilon (green; Opal™520) and anti-LY75/DEC-205 (red; Opal™570) on human thymus. Panel B: anti-CD3 epsilon 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 epsilon 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 epsilon (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 epsilon 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.

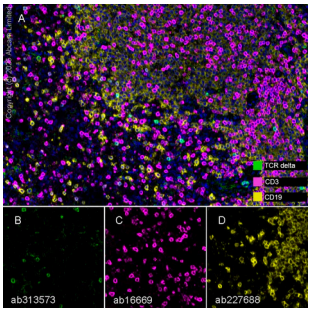


Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Multiplex immunohistochemistry staining of Human colon tissue using rabbit Anti-CD3 epsilon antibody

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 epsilon (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 epsilon 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 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Multiplex immunohistochemistry staining of Human spleen using rabbit Anti-CD3 epsilon antibody

This data was developed using ab313573, the same antibody clone in a different buffer formulation.

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human spleen tissue staining TCR delta with ab313573 at a 1/500 dilution, ab16669 anti-CD3 used at 1/100 dilution and ab227688 anti-CD19 used at a 1/100 dilution.

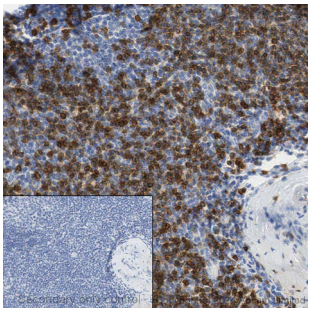
Panel A: merged staining of anti-TCR delta (green; Opal™520), anti-CD3 (magenta; Opal™570) and anti-CD19 (yellow; Opal™690) on human spleen.
Panel B: anti-TCR delta staining immune cells in human spleen.
Panel C: anti-CD3 staining T lymphocytes in human spleen.
Panel D: anti-CD19 staining B lymphocytes in human spleen.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab313573, ab16669 and ab227688 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.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.



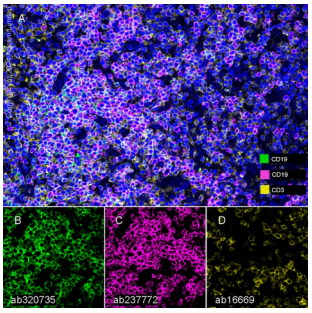
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human tonsil using rabbit Anti-CD3 epsilon antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling CD3 with ab205228 at a concentration of 0.01µg/ml. 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.

ab205228 Anti-CD3 antibody [SP7] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

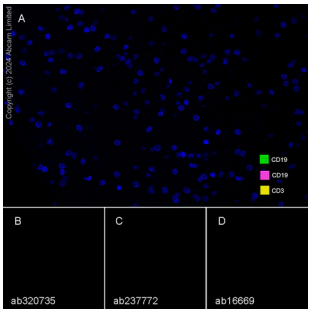
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual).



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

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

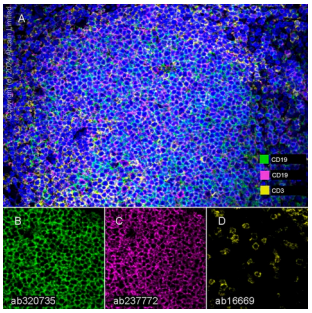
Panel A: merged staining of anti-CD19 (green; Opal™520), anti-CD19 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on human Hodgkin's lymphoma. Panel B: anti-CD19 staining the B lymphocytes in human Hodgkin's lymphoma. Panel C: anti-CD19 staining the B lymphocytes in human Hodgkin's lymphoma. Panel D: anti-CD3 staining the T lymphocytes in human Hodgkin's lymphoma. Nuclear DNA was labeled with DAPI (shown in blue). The section was incubated in three rounds of staining: in the order of ab320735 at 1/2000 dilution, ab237772 at 1/5000 dilution and ab16669 at 1/150 dilution for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The secondary used was Opal Polymer HRP Ms + Rb. 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 epsilon 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-CD19 (green; Opal™520), anti-CD19 (green; Opal™570) and anti-CD3 (yellow; Opal™690) on human cerebrum. Panel B: anti-CD19 showed no staining in human cerebrum. Panel C: anti-CD19 showed no staining in human cerebrum. Panel D: anti-CD3 showed no staining in human cerebrum. Nuclear DNA was labeled with DAPI (shown in blue). The section was incubated in three rounds of staining: in the order of ab320735 at 1/2000 dilution, ab237772 at 1/5000 dilution and ab16669 at 1/150 dilution for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The secondary used was Opal Polymer HRP Ms + Rb. 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 epsilon 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-CD19 (green; Opal™520), anti-CD19 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on human tonsil. Panel B: anti-CD19 staining the B lymphocytes in human tonsil. Panel C: anti-CD19 staining the B lymphocytes in human tonsil. Panel D: anti-CD3 staining the T lymphocytes in human tonsil. Nuclear DNA was labeled with DAPI (shown in blue). The section was incubated in three rounds of staining: in the order of ab320735 at 1/2000 dilution, ab237772 at 1/5000 dilution and ab16669 at 1/150 dilution for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The secondary used was Opal Polymer HRP Ms + Rb. 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.

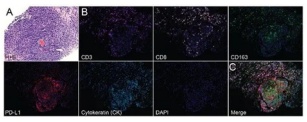
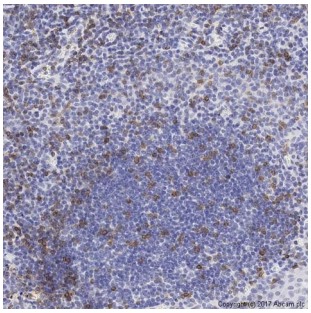


Image from Graff JN et al.,Oncotarget 7(33), 52810 - 52817. Fig 2,; doi: 10.18632/oncotarget.10547. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/3.0/.

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

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

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 epsilon (ab16669), CD8 (ab101500), CD163, PD-L1, cytokeratin (CK), DAPI and C) merged. H+E stating at 20X magnification; multi-spectral images 200X magnification.



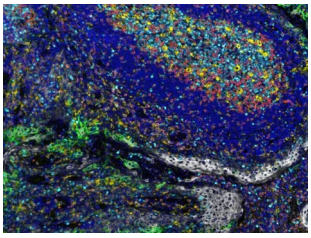
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

This data was developed using ab16669, the same antibody clone in a different buffer formulation. IHC image of CD3 epsilon 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.



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

CD3 epsilon Multiplex immunohistochemistry staining using rabbit Anti-CD3 epsilon antibody

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 epsilon (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.

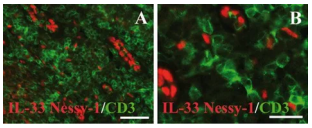


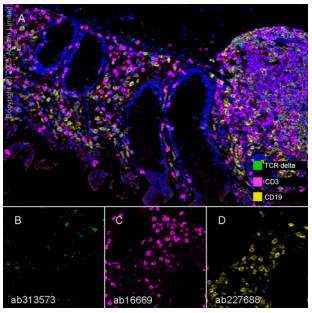
Image from Moussion C et al., PLoS One. 2008 Oct 6;3(10):e3331. Fig 2.; doi:10.1371/journal.pone.0003331; October 6, 2008, PLoS ONE 3(10): e3331.

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

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

This data was developed using ab16669, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of Human tonsil tissue, staining CD3 epsilon (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.



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

CD3 epsilon Multiplex immunohistochemistry staining of Human colon using rabbit Anti-CD3 epsilon antibody

This data was developed using ab313573, the same antibody clone in a different buffer formulation.

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human spleen tissue staining TCR delta with ab313573 at a 1/500 dilution, ab16669 anti-CD3 used at 1/100 dilution and ab227688 anti-CD19 used at a 1/100 dilution.

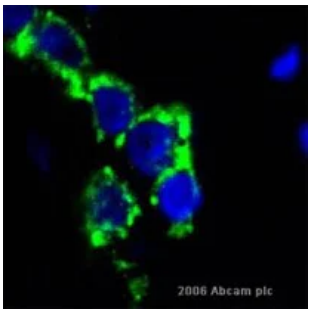
Panel A: merged staining of anti-TCR delta (green; Opal™520), anti-CD3 (magenta; Opal™570) and anti-CD19 (yellow; Opal™690) on human colon.
Panel B: anti-TCR delta staining immune cells in human colon.
Panel C: anti-CD3 staining T lymphocytes in human colon.
Panel D: anti-CD19 staining B lymphocytes in human colon.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab313573, ab16669 and ab227688 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.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins..



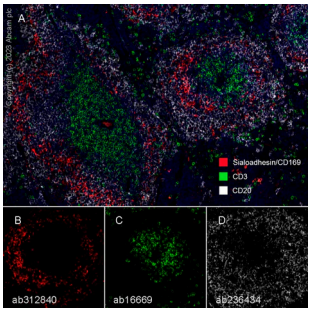
This image is courtesy of a customer review submitted by Dr Mal Niladri.

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

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

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).

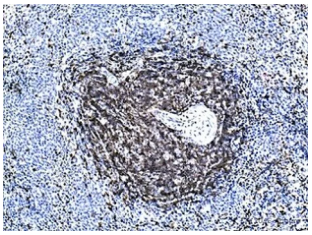
Myocardial infarction was produced in a rat model following the ligation of the left anterior descending (LAD) coronary artery. Tissue was harvested 6 w following infarct, fixed with Histochoice for 72 hr, paraffin sectioned and the slide was then baked prior to CD3 epsilon staining. ab16669 at 1/200 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 epsilon 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 epsilon 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 epsilon localization is predominantly membrane based and to a certain extent intracytoplasmic.



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

CD3 epsilon Multiplex immunohistochemistry staining of Mouse spleen using rabbit Anti-CD3 epsilon antibody

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 epsilon 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 epsilon (green; Opal™520) and anti-CD20 (gray; Opal™570) on mouse spleen. Panel B: anti-Sialoadhesin/CD169 stained on macrophages. Panel C: anti-CD3 epsilon 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.



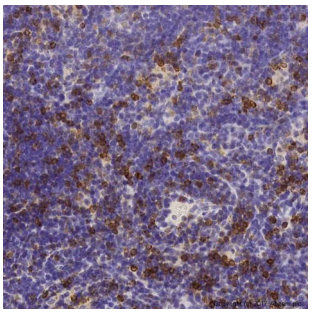
This image is courtesy of a customer review submitted by Carl Hobbs

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

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

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 epsilon 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.

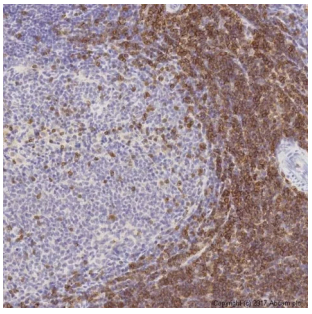


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

This data was developed using ab16669, the same antibody clone in a different buffer formulation. IHC image of CD3 epsilon 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.

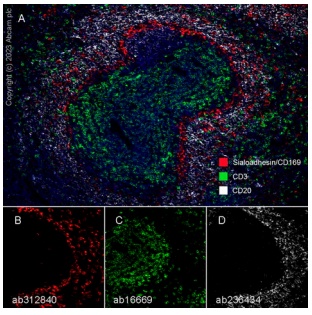


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

This data was developed using ab16669, the same antibody clone in a different buffer formulation. IHC image of CD3 epsilon 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.

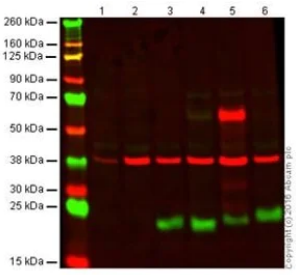


Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Multiplex immunohistochemistry staining of Rat spleen using rabbit Anti-CD3 epsilon antibody

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 epsilon 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 epsilon (green; Opal™520) and anti-CD20 (gray; Opal™570) on rat spleen. Panel B: anti-Sialoadhesin/CD169 stained on macrophages. Panel C: anti-CD3 epsilon 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.



Western blot - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

CD3 epsilon Western blot staining using rabbit Anti-CD3 epsilon antibody

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 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.

All lanes:
Western blot - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228) at 1/25 dilution

Lane 1:
THP1 whole cell lysate (-ve control) at 15 µg

Lane 2:
Raji whole cell lysate (-ve control) at 15 µg

Lane 3:
Jurkat whole cell lysate at 15 µg

Lane 4:
Human Thymus tissue lysate at 15 µg

Lane 5:
Mouse Thymus tissue lysate at 15 µg

Lane 6:
Rat Thymus tissue lysate at 15 µg

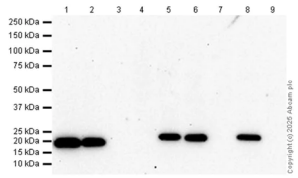
Secondary

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

Developed using the ECL technique.

Predicted band size: 19 kDa

Observed band size: 23 kDa



Western blot - Anti-CD3 epsilon antibody [SP7] - BSA and Azide free (ab205228)

This data was developed using ab16669, the same antibody clone in a different buffer formulation.
Negative control: U-937, Raji, brain.

All lanes:
Western blot - Anti-CD3 epsilon antibody [SP7] (ab16669) at 1/1000 dilution

Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

Lane 2:
MOLT-4 (Human lymphoblastic leukemia T lymphoblast) whole cell lysate at 20 µg

Lane 3:
U-937 (Human histiocytic lymphoma monocyte) whole cell lysate at 20 µg

Lane 4:
Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate at 20 µg

Lane 5:
EL4.IL-2 (mouse lymphoma T lymphocyte) whole cell lysate at 20 µg

Lane 6:
Mouse thymus lysate at 20 µg

Lane 7:
Mouse brain lysate at 20 µg

Lane 8:
Rat thymus lysate at 20 µg

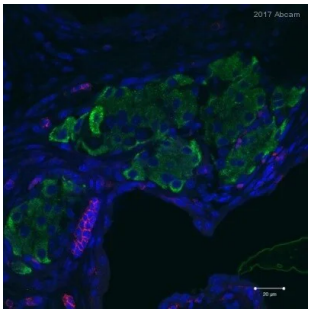
Lane 9:
Rat brain lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 23 kDa

Exposure time: 180s



This image is courtesy of a customer review submitted by Ying Li.

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

This data was developed using ab16669, the same antibody clone in a different buffer formulation. ab16669 staining CD3 epsilon 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.

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