abcam

ab16669

Anti-CD3 epsilon antibody [SP7]

Anti-CD3 epsilon antibody [SP7] (ab16669) is a rabbit monoclonal antibody detecting CD3 epsilon in **Western Blot, Flow Cytometry (Intra), IHC-P, mIHC**. Suitable for **Human, Mouse, Rat**.

- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency
- Over 720 publications
- Trusted since 2005

Lab Essentials | Recombinant | RabMAb | Advanced Validation | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	SP7
Purification technique	Affinity purification Protein A
Concentration	0.009 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

mIHC

Tested

Species	Human
Dilution info	1/100 - 1/2000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Mouse
Dilution info	1/100 - 1/500
Notes	-

Species	Rat
Dilution info	1/100 - 1/500
Notes	-

Predicted

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

IHC-P

Tested

Species	Mouse
Dilution info	1/150
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/150
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.

Species	Human
Dilution info	1/150

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100
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	Rabbit, Horse, Chicken, Cow, Cat, Dog, Pig, Baboon, Woodchuck
Dilution info	-
Notes	-

WB

Tested

Species	Mouse

Dilution info	1/25 - 1/1000
Notes	-

Species	Rat
Dilution info	1/25 - 1/1000
Notes	-

Species	Human
Dilution info	1/25 - 1/1000
Notes	-

Predicted

Species	Rabbit, Horse, Chicken, Cow, Cat, Dog, Pig, Baboon, Woodchuck
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	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Anti-CD3 epsilon antibody [SP7] (ab16669) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), IHC-P, WB and mIHC.

Anti-CD3 epsilon antibody [SP7] (ab16669) was first used in a scientific publication in 2008 and has been cited over 722 times in peer reviewed journals. It's performance in IHC in human, mouse and rat samples is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-CD3 epsilon antibody [SP7] (ab16669) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

Anti-CD3 epsilon antibody [SP7] (ab16669) has 79 independent reviews from customers.

Anti-CD3 epsilon antibody [SP7] (ab16669) specifically detects CD3 epsilon (UniProt ID: P07766; Molecular weight: 21kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL, 500 µL and 1 mL).

Conjugation-ready, carrier free format available for antibody clone SP7 - ab205228.

Anti-CD3 epsilon antibody [SP7] (ab16669) is a clone from the portfolio of Spring Bioscience (Roche) SP clones which have been optimised for immunohistochemistry (IHC).

Top cited recombinant antibody on the market with >988 citations. SP7 has received >79 reviews. CD3 epsilon (CD3ε) is a crucial component of the T-cell receptor (TCR) complex. It plays a key role in T-cell activation and signal transduction by coupling antigen recognition to intracellular signalling pathways.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CD3 epsilon also known as CD3e or CD3 epsilon protein is a component of the T-cell receptor (TCR) complex which plays a role in antigen recognition. The CD3 epsilon protein has a molecular mass of approximately 20 kDa. This protein is expressed in T cells across the thymus and peripheral lymphoid organs. It forms part of the CD3 complex which also includes CD3 gamma CD3 delta and CD3 zeta chains. This complex associates with the TCR alpha and beta chains facilitating critical signaling events for T-cell activation and development.
Biological function summary	CD3 epsilon functions as part of the TCR-CD3 complex essential for the immune response. It acts as a signal transducer initiating intracellular signaling upon antigen engagement. This signaling results in T-cell activation proliferation and differentiation. The CD3 complex including CD3 epsilon is a necessary participant in thymic selection a process ensuring the development of functional and self-tolerant T-cells. The specificity and function of CD3 epsilon make it a target for monoclonal antibodies such as anti-CD3 used for modulating T-cell functions.
Pathways	Several immune-regulatory pathways involve CD3 epsilon including the TCR signaling pathway and NF-κB pathway. CD3 epsilon works in conjunction with proteins such as CD28 and LCK to transmit signals from the TCR complex to downstream pathways. These pathways lead to the activation of transcription factors like NF-κB and AP-1 promoting cytokine production and T-cell proliferation. CD3 epsilon also interfaces with other pathways through interaction with zeta-chain-associated protein kinase 70 (ZAP-70) which is important for signal transduction.
Associated diseases and disorders	Autoimmune diseases and certain types of cancer relate to CD3 epsilon activity. Aberrant functioning or expression levels of the CD3 epsilon protein can contribute to autoimmune conditions such as systemic lupus erythematosus due to improper T-cell activation. Additionally CD3 epsilon is targeted by therapeutic antibodies like muromonab used in prevention of acute organ transplant rejection. Altered pathways involving CD3 epsilon and associated proteins like CD3 zeta also have implications in leukemia highlighting the significance of this protein in both normal immune function and pathology.

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.

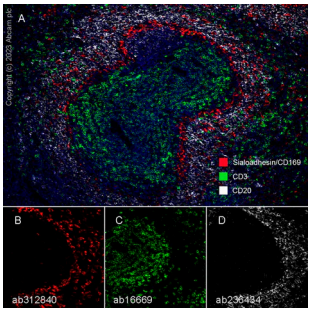
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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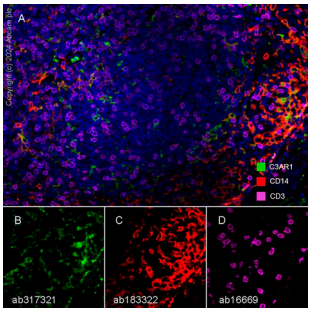
30 product images



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

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

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.



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

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human tonsil tissue staining C3a R with ab317321 at a 1:1000 (0.52 ug/ml) dilution, ab183322 anti-CD14 used at 1:100 (1.22 ug/ml) dilution and ab16669 anti-CD3 epsilon used at a 1:150 (0.06 ug/ml) dilution.

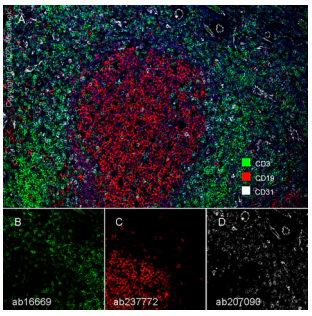
Panel A: merged staining of anti-C3AR1 (green; Opal™520) anti-CD14 (red; Opal™570) anti-CD3 epsilon (magenta; Opal™690) on human tonsil.
Panel B: anti-C3AR1 stained on immune cells.
Panel C: anti-CD14 stained on macrophages.
Panel D: anti-CD3 epsilon stained on T lymphocytes.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab317321, ab183322 and ab16669 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.

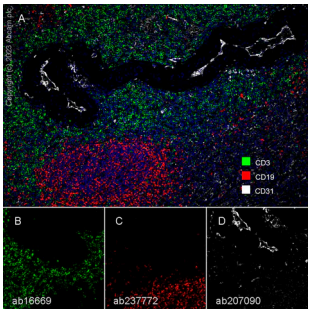
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0 Epitope Retrieval Solution2) for 20 mins.



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

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

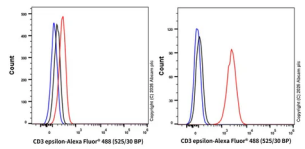
Panel A: merged staining of anti-CD31 (gray; Opal™690), anti-CD3 epsilon (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human tonsil. Secondary antibody was Opal Polymer HRP Ms + Rb, nuclear counterstain was DAPI. Panel B: anti-CD3 epsilon stained 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 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



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

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

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] (ab16669)

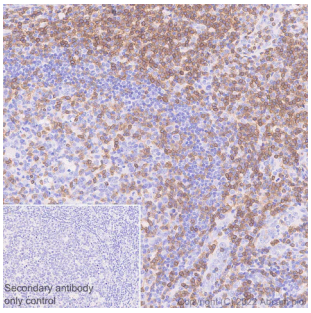
CD3 epsilon Flow Cytometry (Intracellular) staining using rabbit Anti-CD3 epsilon antibody

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] (ab16669)

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

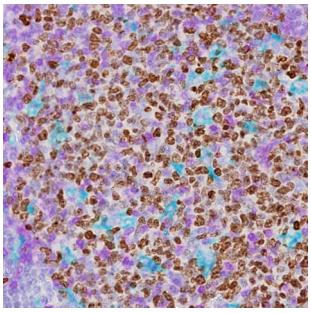
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD3 epsilon with ab16669 at 1/150 dilution.
Positive staining on human tonsil

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.

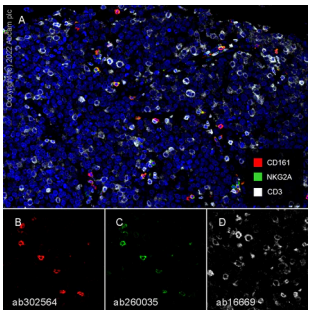
Heat mediated antigen retrieval was performed with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins.



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

CD3 epsilon Multiplex immunohistochemistry staining of human tonsil tissue using rabbit Anti-CD3 epsilon antibody

Chromogenic multiplex immunohistochemical staining of FFPE normal human tonsil tissue. ab16667 anti-Ki67 DAB chromogen. ab16669 anti-CD3 epsilon purple chromogen and ab192847 anti-CD68 teal chromogen plus haematoxylin II counterstain. Chromogenic immunostaining was performed on a Roche Ventana Benchmark Ultra instrument. The section was deparaffinised and incubated with CC1 solution for 24min 100°C. Following this with 3 rounds of staining in the order of ab16667 (1/500 dilution), ab192847 (1/4000 dilution) and ab16669 (1/1000 dilution). Between rounds of staining antibody denaturation was conducted using Ultra CC2 solution for 8min at 100oC to avoid cross reactivity. Signal was developed with anti-rabbit HQ followed by anti-HQ HRP coupled with Chromomap DAB kit Discovery purple or Discovery teal chromogens and haematoxylin II counterstain.

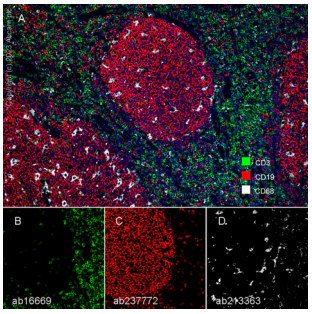


Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [SP7] (ab16669)

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

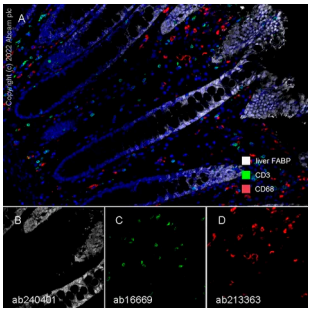
Multiplex immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded sections of human NK-T lymphoma tissue labeling CD-161 with ab302564 at 1:100 dilution (4.96 µg/mL) colored in red (Opal™570, red). Panel A: merged staining of anti-CD3 (ab16669, grey; Opal™690), anti-CD161(ab302564, red; Opal™570) and anti-NKG2A (ab260035, green; Opal™520) on human NK-T lymphoma. Panel B: anti-CD161 staining only. ab302564 (Opal™ 570, Red) at 1/100 dilution (4.96 µg/mL) Panel C: anti-NKG2A staining only. ; ab260035, (Opal™ 520, Green) NKG2A at 1/2000

dilution (0.283 µg/mL) Panel D: anti-CD3 staining only. CD3 staining only; ab16669 (Opal™ 690, Gray) at 1/2000 dilution (0.0675 µg/mL) The section was incubated in three rounds of staining: in the order of ab16669 for 30 mins, ab302564 for 30 mins and ab260035 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Nuclear stained with DAPI. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used. 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] (ab16669)

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] (ab16669)

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.

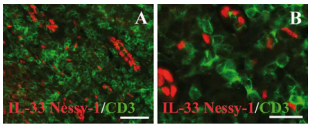


Image from Moussion C et al., PLoS One. 2008 Oct 6;3(10):e3331. Fig 2.; doi:10.1371/journal.pone.0003331; October 6, 2008, PLoS ONE 3(10): e3331.

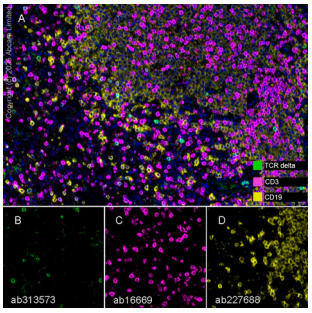
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [SP7] (ab16669)

This image was generated using a previous batch manufactured using hybridoma production method.

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] (ab16669)

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

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.

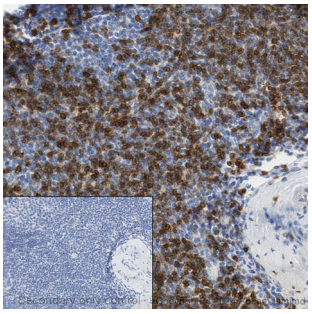
Panel A: merged staining of anti-TCR delta (green; Opal™520), anti-CD3 (magenta; Opal™570) and anti-CD19 (yellow; Opal™690) on human spleen.
Panel B: anti-TCR delta staining immune cells in human spleen.
Panel C: anti-CD3 staining T lymphocytes in human spleen.
Panel D: anti-CD19 staining B lymphocytes 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] (ab16669)

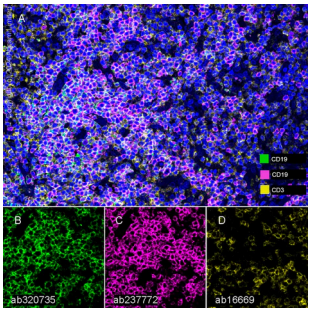
CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human tonsil using rabbit Anti-CD3 epsilon antibody

This data was developed using ab205228, the same antibody clone in a different buffer formulation.

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.

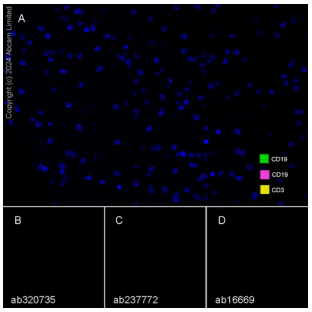
ab205228 Anti-CD3 antibody [SP7] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

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).



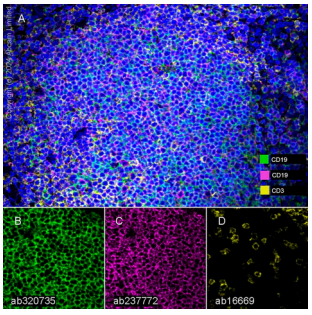
Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [SP7] (ab16669)

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.



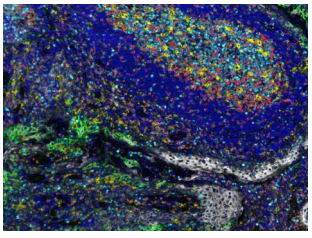
Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [SP7] (ab16669)

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] (ab16669)

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.



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

CD3 epsilon Multiplex immunohistochemistry staining of human tonsil tissue using rabbit Anti-CD3 epsilon antibody

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).

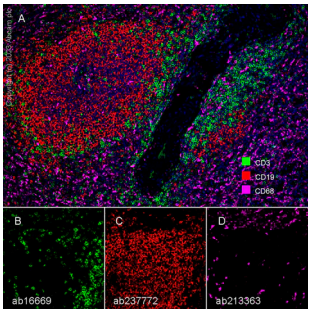
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 7-color automation IHC kit (NEL821001KT Akoya Biosciences®).

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.

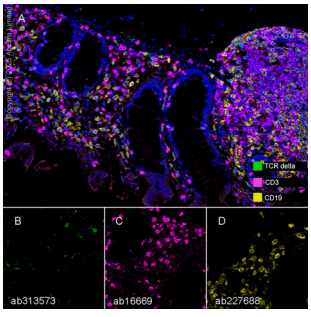
Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra Polaris.



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

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

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] (ab16669)

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

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.

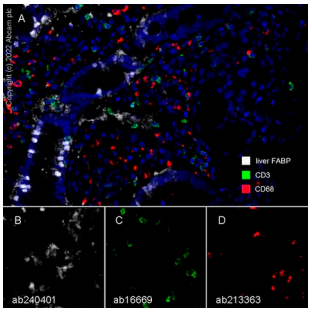
Panel A: merged staining of anti-TCR delta (green; Opal™520), anti-CD3 (magenta; Opal™570) and anti-CD19 (yellow; Opal™690) on human colon.
Panel B: anti-TCR delta staining immune cells in human colon.
Panel C: anti-CD3 staining T lymphocytes in human colon.
Panel D: anti-CD19 staining B lymphocytes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

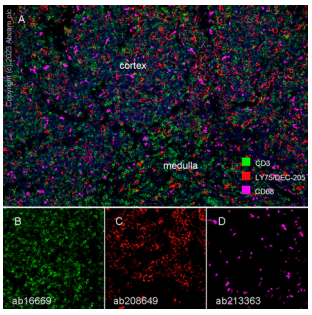
Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins..



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

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

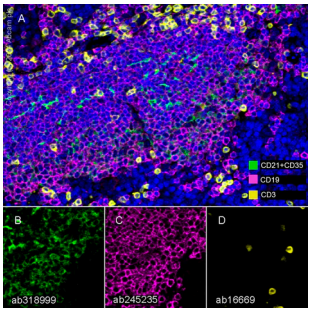
Fluorescence multiplex immunohistochemical analysis of the human duodenum (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-liver FABP (ab240401 gray; Opal™690), anti-CD3 epsilon (ab16669 green; Opal™520) and anti-CD68 (ab213363 red; Opal™570) on human duodenum. Panel 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.



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

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

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] (ab16669)

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse spleen tissue staining CD21+CD35 with ab318999 at a 1:2000 (0.255 ug/ml) dilution, ab245235 anti-CD19 used at 1:1000 (0.444 ug/ml) dilution and ab16669 anti-CD3 used at a 1:150 (0.06 ug/ml) dilution.

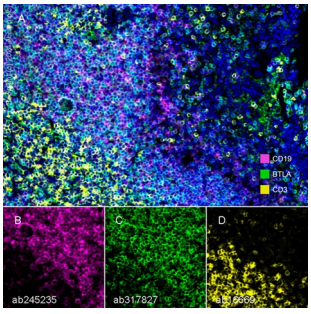
Panel A: merged staining of anti-CD21+CD35 (green; Opal™520), anti-CD19 (magenta; Opal™690) and anti-CD3 (yellow; Opal™570) on mouse spleen.
Panel B: anti-CD21+CD35 staining B lymphocytes in mouse spleen.
Panel C: anti-CD19 staining B lymphocytes in mouse spleen.
Panel D: anti-CD3 staining T lymphocytes in mouse spleen.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab318999, ab245235 and ab16669 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 with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



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

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse spleen tissue staining CD19 with ab245235 at a 1:1000 (0.44 ug/ml) dilution, ab317827 anti-BTLA used at 1:100 (4.92 ug/ml) dilution and ab16669 anti-CD3 used at a 1:150 (0.06 ug/ml) dilution.

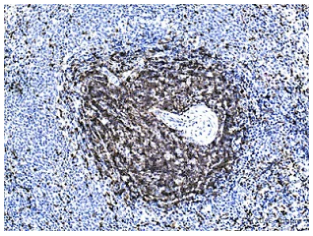
Panel A: merged staining of anti-CD19 (magenta; Opal™690), anti-BTLA (green; Opal™520) and anti-CD3 (yellow; Opal™570) on mouse spleen.
Panel B: anti-CD19 staining B lymphocytes in mouse spleen.
Panel C: anti-BTLA staining B lymphocytes and T lymphocytes in mouse spleen.
Panel D: anti-CD3 staining T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

The section was incubated in three rounds of staining: in the order of ab245235, ab317827 and ab16669 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 with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



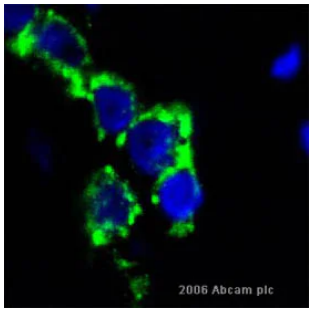
This image is courtesy of a customer review submitted by Carl Hobbs

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

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

This image was generated using a previous batch manufactured using hybridoma production method.

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.



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

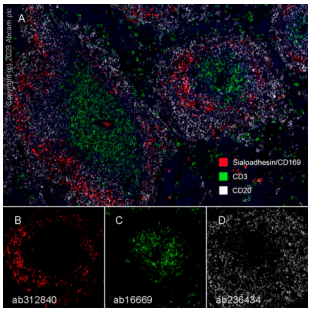
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [SP7] (ab16669)

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

This image was generated using a previous batch manufactured using hybridoma production method.

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

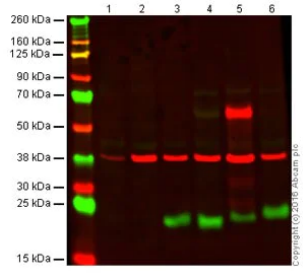
Myocardial infarction was produced in a rat model following the ligation of the left anterior descending (LAD) coronary artery. Tissue was harvested 6 w following infarct, fixed with Histochoice for 72 hr, paraffin sectioned and the slide was then baked prior to CD3 epsilon staining. ab16669 at 1/200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

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

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.



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

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

This image was generated using a previous batch manufactured using hybridoma production method.

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] (ab16669) 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

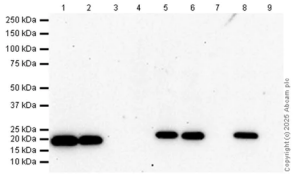
Secondary

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

Performed under reducing conditions.

Predicted band size: 19 kDa

Observed band size: 23 kDa



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

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

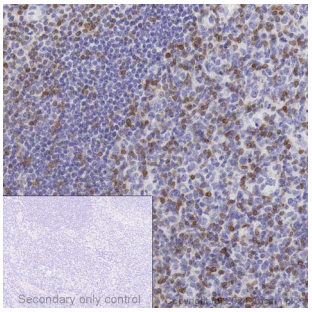
Lane 9:
Rat brain lysate at 20 µg

Secondary

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

Observed band size: 23 kDa

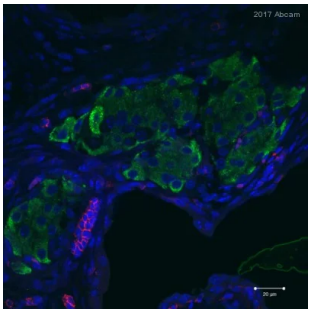
Exposure time: 180s



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

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

Immunohistochemical analysis of formalin fixed paraffin (FFPE) embedded tonsil labelling CD3 epsilon with ab16669 at a dilution of 1/600. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an ChromoMap DAB (RUO) IHC Detection Kit with anti rabbit HQ and anti HQ HRP. Heat mediated antigen retrieval was conducted for 24 min with DISCOVERY cell conditioning solution (CC1) 100°C, pH 8.5. ab16669 was incubated at 37°C for 16 min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



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

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

This image was generated using a previous batch manufactured using hybridoma production method.

ab16669 staining CD3 epsilon 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.

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