abcam

ab237721

Anti-CD3 epsilon antibody [CAL57]

Anti-CD3 epsilon antibody [CAL57] (ab237721) is a rabbit monoclonal antibody detecting CD3 epsilon in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, IHC-Fr, mIHC**. Suitable for **Human, Mouse, Rat**.

- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency

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

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.5 Preservative: 0.05% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	CAL57
Purification technique	Affinity purification Protein A
Concentration	0.501 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purity >99%.

Reactivity data

mIHC

Tested

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

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

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

IHC-P

Tested

Species	Mouse
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

Expected

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

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

IHC-Fr

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
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 duration	1-2 weeks
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

What is this antibody validated in?
Anti-CD3 epsilon antibody [CAL57] (ab237721) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunohistochemistry (IHC-Fr), Multiplex IHC (mIHC) in Human, Mouse, Rat samples.

What is the molecular weight of CD3 epsilon?
Anti-CD3 epsilon [CAL57] (ab237721) specifically detects a band for CD3 epsilon (UniProt: P22646) at a molecular weight of 23kDa.

Trusted by the scientific community
Anti-CD3 epsilon [CAL57] (ab237721) was first used in a scientific publication in 2018 and has been cited over 10 times in peer-reviewed journals.

Other related products
We have a range of other formats of antibody clone [CAL57] also available for your convenience: ab237721, Carrier free - ab251607

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

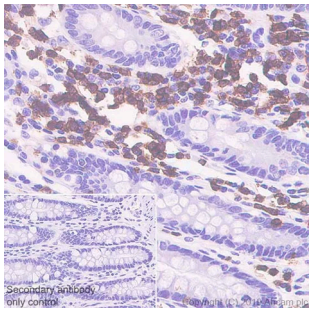
We do not recommend this combination. It is not covered by our product promise.

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.

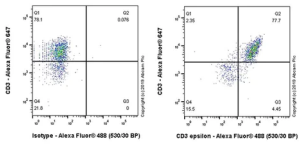
40 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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

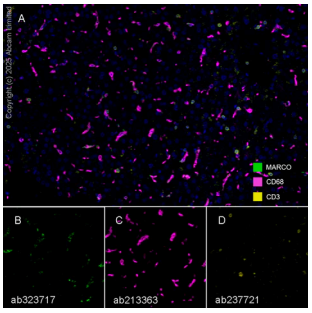
Immunohistochemical analysis of paraffin-embedded human colon tissue labeling CD3 epsilon with ab237721 at 1/2000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the T lymphocytes in the human colon. The section was incubated with ab237721 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Flow Cytometry (Intracellular) - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized human peripheral blood mononuclear cell (PBMC) labeling CD3 epsilon with ab237721 at 1/500 (right panel) compared with a Rabbit monoclonal IgG (ab172730) (left panel). Goat anti rabbit IgG (Alexa Fluor® 488, ab150097), at 1/5000 dilution was used as the secondary antibody.
Cells were surface stained with anti-CD3 conjugated to Alexa Fluor® 647. Then fixed with 2% PFA for 10min and intracellularly stained with rabbit IgG (Left) or ab237721 (Right). They recognize the same cell population.



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human liver tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab213363 anti-CD68 used at 1:500 (1.26 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

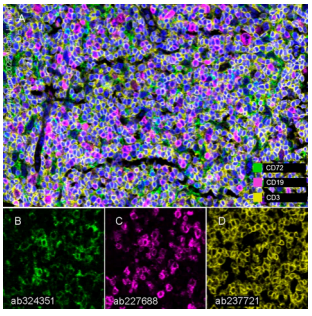
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD68 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on human liver.
Panel B: anti-MARCO staining macrophages in human liver.
Panel C: ant-CD68 staining macrophages in human liver.
Panel D: ant-CD3 staining T lymphocytes in human liver.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab323717, ab213363 and ab237721 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 [CAL57] (ab237721)

CD3 epsilon Multiplex immunohistochemistry staining of Human Hodgkin's lymphoma using rabbit Anti-CD3 epsilon antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human hodgkin's lymphoma tissue staining CD72 with ab324351 at a 1:500 (1.018 ug/ml) dilution, ab227688 anti-CD19 used at 1:100 (0.15 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

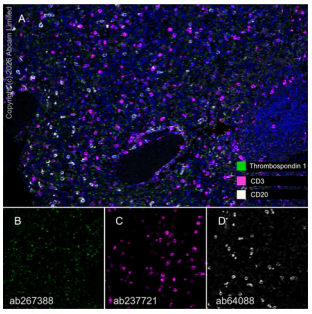
Panel A: merged staining of anti-CD72 (green; Opal™520), anti-CD19 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on human Hodgkin's lymphoma.
Panel B: anti-CD72 staining immune cells in human Hodgkin's lymphoma.
Panel C: ant-CD19 staining B lymphocytes in human Hodgkin's lymphoma.
Panel D: ant-CD3 staining 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 ab324351, ab227688 and ab237721 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 [CAL57] (ab237721)

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 Thrombospondin 1 with ab267388 at a 1/5000 dilution, ab237721 anti-CD3 used at 1/2000 dilution and ab64088 anti-CD20 used at a 1/100 dilution.
Panel A: merged staining of anti-Thrombospondin 1 (green; Opal™520), anti-CD3 (magenta; Opal™690) and anti-CD20 (gray; Opal™570) on human spleen.

Panel B: anti-Thrombospondin 1 staining megakaryocytes or platelet in human spleen.

Panel C: anti-CD3 staining T lymphocytes in human spleen.

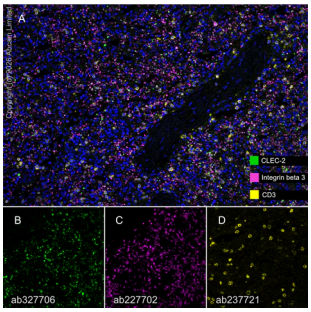
Panel D: anti-CD20 staining B lymphocytes in human spleen.

Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab267388, ab237721 and ab64088 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.

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 [CAL57] (ab237721)

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 with ab327706 at a 1:100 (4.87 µg/ml) dilution, ab227702 anti-Integrin beta 3 used at 1:100 (0.40 µg/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 µg/ml) dilution.

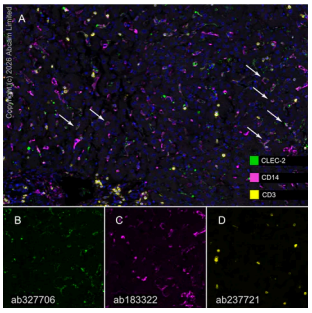
Panel A: merged staining of anti-CLEC-2 (green; Opal™520), anti-Integrin beta 3 (magenta; Opal™690) and anti-CD3 (yellow; Opal™570) on human spleen.
Panel B: anti-CLEC-2 staining platelets in human spleen.
Panel C: anti-Integrin beta 3 staining platelets in human spleen.
Panel D: anti-CD3 staining T 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 ab327706, ab227702 and ab237721 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 [CAL57] (ab237721)

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human liver tissue staining with ab327706 at a 1:100 (4.87 µg/ml) dilution, ab183322 anti-CD14 used at 1:100 (01.22 µg/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 µg/ml) dilution.

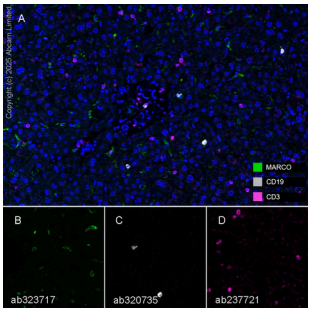
Panel A: merged staining of anti-CLEC-2 (green; Opal™520), anti-CD14 (magenta; Opal™690) and anti-CD3 (yellow; Opal™570) on human spleen.
Panel B: anti-CLEC-2 staining platelets in human spleen.
Panel C: anti-CD14 staining monocytes/macrophages in human spleen.
Panel D: anti-CD3 staining T lymphocytes 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 ab327706, ab183322 and ab237721 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 [CAL57] (ab237721)

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human liver tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab320735 anti-CD19 used at 1:2000 (0.255 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

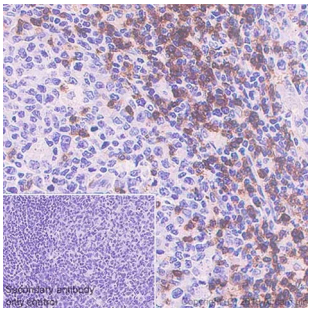
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD19 (gray; Opal™570) and anti-CD3 (magenta; Opal™690) on human liver.
Panel B: anti-MARCO staining macrophages in human liver.
Panel C: ant-CD19 staining B lymphocytes in human liver.
Panel D: ant-CD3 staining T lymphocytes in human liver.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab323717, ab320735 and ab237721 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.

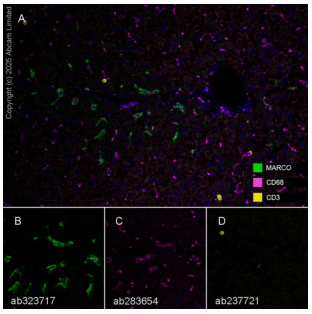
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD3 epsilon with ab237721 at 1/2000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the T lymphocytes in the human tonsil. The section was incubated with ab237721 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

CD3 epsilon Multiplex immunohistochemistry staining of rat liver tissue using rabbit Anti-CD3 epsilon antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded rat liver tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab283654 anti-CD68 used at 1:100 (4.43 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

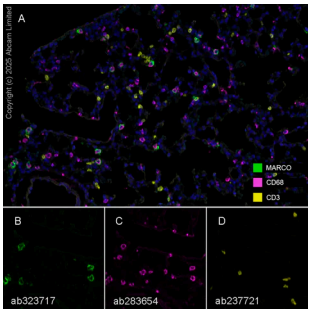
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD68 (magenta; Opal™690) and anti-CD3 (yellow; Opal™570) on rat liver.
Panel B: anti-MARCO staining macrophages in rat liver.
Panel C: ant-CD68 staining macrophages in rat liver.
Panel D: ant-CD3 staining T lymphocytes in rat liver.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab323717, ab283654 and ab237721 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 [CAL57] (ab237721)

CD3 epsilon Multiplex immunohistochemistry staining of mouse lung tissue using rabbit Anti-CD3 epsilon antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse lung tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab283654 anti-CD68 used at 1:100 (4.43 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

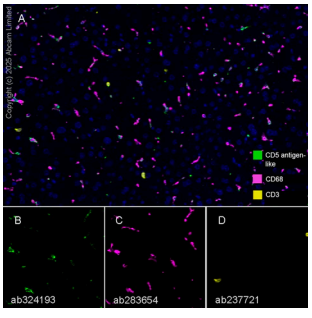
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD68 (magenta; Opal™690) and anti-CD3 (yellow; Opal™570) on mouse lung.
Panel B: anti-MARCO staining macrophages in mouse lung.
Panel C: ant-CD68 staining macrophages in mouse lung.
Panel D: ant-CD3 staining T lymphocytes in mouse lung.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab323717, ab283654 and ab237721 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 [CAL57] (ab237721)

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

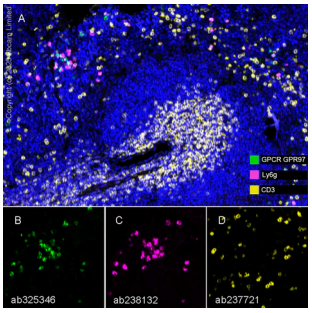
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse liver tissue staining CD5L/CT-2 with ab324193 at a 1:500 (0.958 ug/ml) dilution, ab283654 anti-CD68 used at 1:100 (4.43 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.25 ug/ml) dilution.

Panel A: merged staining of anti-CD5 antigen-like (green; Opal™520), anti-CD68 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on mouse liver.
Panel B: anti-CD5 antigen-like staining macrophages in mouse liver.
Panel C: anti-CD68 staining macrophages in mouse liver.
Panel D: anti-CD3 staining T lymphocytes in mouse liver.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab324193, ab283654 and ab237721 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

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 [CAL57] (ab237721)

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 with ab325346 at a 1:5000 (0.099 µg/ml) dilution, ab238132 anti-Ly6g used at 1:100 (0.29 µg/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 µg/ml) dilution.

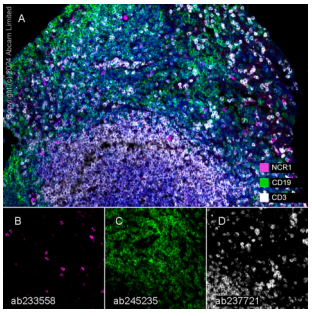
Panel A: anti-GPCR GPR97 (green; Opal™520), anti-Ly6g (magenta; Opal™690) and anti-CD3 (yellow; Opal™570) on mouse spleen.
Panel B: anti-GPCR GPR97 staining granulocytes in mouse spleen.
Panel C: anti-Ly6g staining neutrophils in mouse spleen.
Panel D: anti-CD3 staining T lymphocytes in mouse spleen.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab325346, ab238132 and ab237721 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 [CAL57] (ab237721)

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

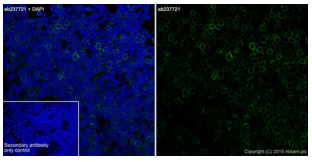
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Mouse lymph node staining of NCR1 with ab233558 at a 1/500 (1.062 µg/ml) dilution, CD19 with ab245235 at 1/1000 (0.444 µg/ml) dilution and CD3 with ab237721 at 1/2000 (0.264 µg/ml) dilution.

Panel A: merged staining of anti-NCR1 (magenta; Opal™690), anti-CD19 (green; Opal™520) and anti-CD3 (gray; Opal™570) on mouse lymph node.
Panel B: anti-NCR1 staining natural killer cells in mouse lymph node.
Panel C: anti-CD19 staining B lymphocytes in mouse lymph node..
Panel D: anti-CD3 staining T lymphocytes in mouse lymph node.
Nuclear DNA was labelled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab233558, ab245235 and ab237721 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.

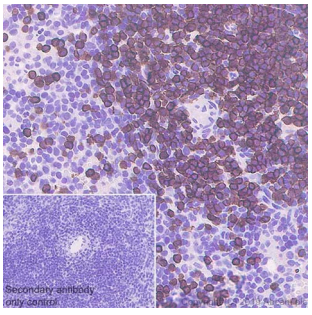
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0 epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Frozen sections) - Anti-CD3 epsilon antibody [CAL57] (ab237721)

CD3 epsilon Immunohistochemistry (Frozen sections) staining using rabbit Anti-CD3 epsilon antibody

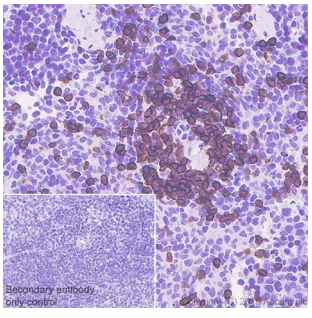
Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse thymus tissue labeling CD3 epsilon with ab237721 at 1/500 dilution (green), followed by ab150077 AlexaFluor®488 Goat anti-Rabbit secondary at a 1/1,000 dilution. Positive staining on T cell surface (PMID: 8490660) is observed. Counterstained with DAPI (blue). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab150077 AlexaFluor®488 Goat anti-Rabbit used at a 1/1,000 dilution. Heat mediated antigen retrieval using sodium citrate buffer (10 mM citrate pH 6.0 and 0.05% Tween-20).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling CD3 epsilon with ab237721 at 1/2000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the T lymphocytes in the mouse spleen. The section was incubated with ab237721 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

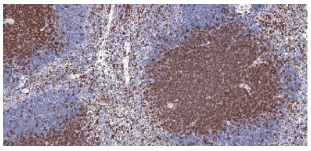


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling CD3 epsilon with ab237721 at 1/2000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the T lymphocytes in the rat spleen. The section was

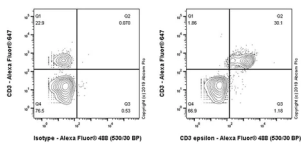
incubated with ab237721 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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

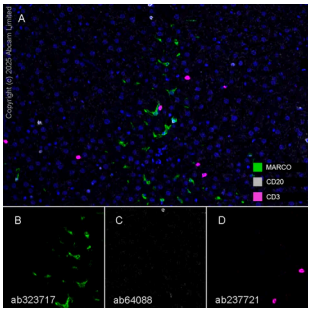
Formalin-fixed, paraffin-embedded mouse spleen tissue stained for CD3 epsilon using ab237721 at 0.5 µg/ml in immunohistochemical analysis.



Flow Cytometry (Intracellular) - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized mouse splenocytes labeling CD3 epsilon with ab237721 at 1/500 (right panel) compared with a Rabbit monoclonal IgG (ab172730) (left panel). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody.
Cells were surface stained with anti-CD3 conjugated to Alexa Fluor® 647. Then fixed with 2% PFA for 10min and intracellularly stained with rabbit IgG (Left) or ab237721 (Right). They recognize the same cell population.



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

CD3 epsilon Multiplex immunohistochemistry staining of rat liver tissue using rabbit Anti-CD3 epsilon antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded rat liver tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab64088 anti-CD20 used at 1:100 (2.91 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

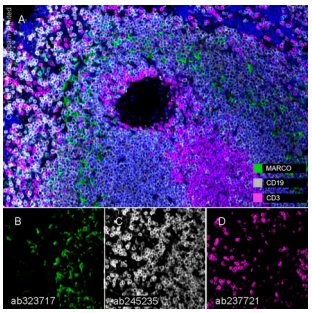
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD20 (gray; Opal™690) and anti-CD3 (magenta; Opal™570) on rat liver.
Panel B: anti-MARCO staining macrophages in rat liver.
Panel C: ant-CD20 staining B lymphocytes in rat liver.
Panel D: ant-CD3 staining T lymphocytes in rat liver.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab323717, ab64088 and ab237721 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 [CAL57] (ab237721)

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse spleen tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab245235 anti-CD19 used at 1:1000 (0.22 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

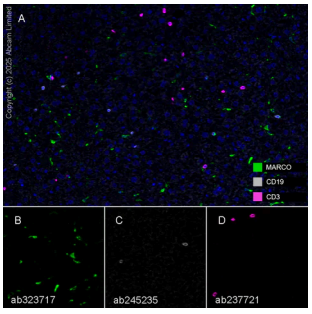
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD19 (gray; Opal™690) and anti-CD3 (magenta; Opal™570) on mouse spleen.
Panel B: anti-MARCO staining macrophages in mouse spleen.
Panel C: ant-CD19 staining B lymphocytes in mouse spleen.
Panel D: ant-CD3 staining T lymphocytes in mouse spleen.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

The section was incubated in three rounds of staining: in the order of ab323717, ab245235 and ab237721 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 [CAL57] (ab237721)

CD3 epsilon Multiplex immunohistochemistry staining of mouse liver tissue using rabbit Anti-CD3 epsilon antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse liver tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab245235 anti-CD19 used at 1:1000 (0.22 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

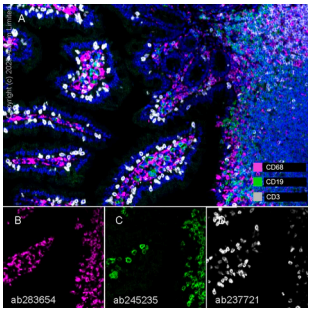
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD19 (gray; Opal™690) and anti-CD3 (magenta; Opal™570) on mouse liver.
Panel B: anti-MARCO staining macrophages in mouse liver.
Panel C: ant-CD19 staining B lymphocytes in mouse liver.
Panel D: ant-CD3 staining T lymphocytes in mouse liver.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

The section was incubated in three rounds of staining: in the order of ab323717, ab245235 and ab237721 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.



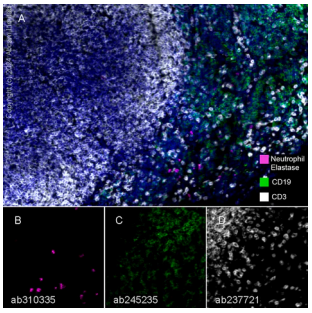
Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse small intestine tissue staining CD68 with ab283654 at a 1/100 dilution, ab245235 anti-CD19 used at 1/1000 dilution and ab237721 anti-CD3 used at a 1/2000 dilution. Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD68 (magenta; Opal™520), anti-CD19 (green; Opal™690) and anti-CD3 (grey; Opal™570) on mouse small intestine.
Panel B: anti-CD68 staining macrophages in mouse small intestine.
Panel C: anti-CD19 staining B lymphocytes in mouse small intestine.
Panel D: anti-CD3 staining T lymphocytes in mouse small intestine.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab283654, ab245235 and ab237721 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 [CAL57] (ab237721)

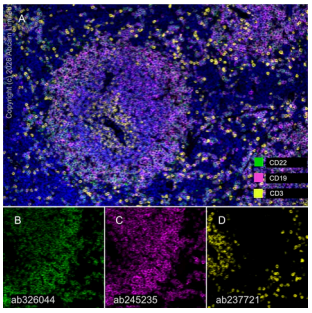
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Mouse lymph node tissue staining Neutrophil Elastase with ab310335 at a 1/500 (1.076 µg/ml) dilution, CD19 with ab245235 at a 1/1000 (0.444 µg/ml) dilution and CD3 with ab237721 at a 1/2000 (0.264 µg/ml) dilution followed by a ready to use secondary antibody Opal Polymer HRP Ms + Rb.

Panel A: merged staining of anti-Neutrophil Elastase (magenta; Opal™690), anti-CD19 (green; Opal™520) and anti-CD3 (gray; Opal™690) on mouse spleen.Panel B: anti-Neutrophil Elastase staining neutrophils 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 ab310335, ab245235 and ab237721 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.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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 with ab326044 at a 1:500 (0.982 µg/ml) dilution, ab245235 anti-CD19 used at 1:1000 (0.479 µg/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 µg/ml) dilution.

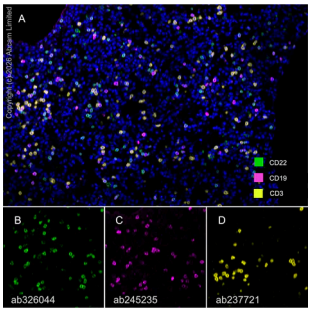
Panel A: anti-CD22 (green; Opal™520), anti-CD19 (magenta; Opal™570), anti-CD3 (yellow; Opal™690) on mouse spleen.
Panel B: anti-CD22 staining B lymphocytes in mouse spleen.
Panel C: anti-CD19 staining B lymphocytes in mouse spleen.
Panel D: anti-CD3 staining on T lymphocytes in mouse spleen.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab326044, ab245235 and ab237721 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 [CAL57] (ab237721)

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse lung tissue staining with ab326044 at a 1:500 (0.982 µg/ml) dilution, ab245235 anti-CD19 used at 1:1000 (0.479 µg/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 µg/ml) dilution.

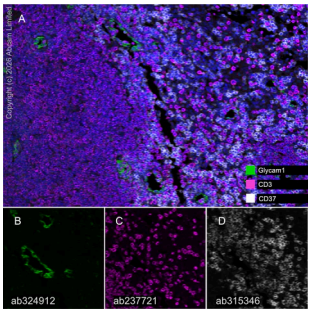
Panel A: anti-CD22 (green; Opal™520), anti-CD19 (magenta; Opal™570), anti-CD3 (yellow; Opal™690) on mouse lung.
Panel B: anti-CD22 staining B lymphocytes in mouse lung.
Panel C: anti-CD19 staining B lymphocytes in mouse lung.
Panel D: anti-CD3 staining on T lymphocytes in mouse lung.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab326044, ab245235 and ab237721 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 [CAL57] (ab237721)

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse lymph node tissue staining Glycam1 with ab324912 at a 1/4000 (0.126 µg/ml) dilution, ab237721 anti-CD3 used at 1/2000 dilution (0.26 µg/ml) and ab315346 anti-CD37 used at 1/2000 dilution (0.257 µg/ml).
Panel A: anti-Glycam1 (green; Opal™520), anti-CD3 (magenta; Opal™690), anti-CD37 (gray; Opal™570) on mouse lymph node.

Panel B: anti-Glycam1 staining HEV (highly endothelial venules) in mouse lymph node.

Panel C: anti-CD3 staining T lymphocytes in mouse lymph node.

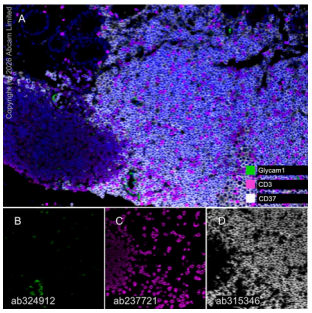
Panel D: anti-CD37 staining B lymphocytes in mouse lymph node.

Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab324912, ab237721 and ab315346 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.

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 [CAL57] (ab237721)

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

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse colon tissue staining Glycam1 with ab324912 at a 1/4000 (0.126 µg/ml) dilution, ab237721 anti-CD3 used at 1/2000 dilution (0.26 µg/ml) and ab315346 anti-CD37 used at 1/2000 dilution (0.257 µg/ml). Panel A: anti-Glycam1 (green; Opal™520), anti-CD3 (magenta; Opal™690), anti-CD37 (gray; Opal™570) on mouse colon.

Panel B: anti-Glycam1 staining HEV (highly endothelial venules) in mouse colon.

Panel C: anti-CD3 staining T lymphocytes in mouse colon.

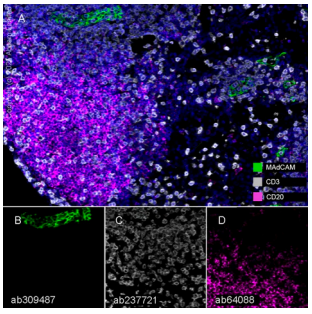
Panel D: anti-CD37 staining B lymphocytes in mouse colon.

Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab324912, ab237721 and ab315346 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.

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 [CAL57] (ab237721)

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

Immunohistochemistry analysis of (Formalin/PFA-fixed paraffin-embedded sections) Rat lymph node tissue labelling MAdCAM1 with ab309487 at 1:100 dilution (B), CD3 epsilon with ab237721at 1:2000 dilution (C) and CD20 with ab64088 at 1:100 dilution (D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-MAdCAM (green; Opal™520), anti-CD3 (gray; Opal™690) and anti-CD20 (magenta; Opal™570) on rat lymph node.

Panel B: anti-MAdCAM staining high endothelial venules in rat lymph node.

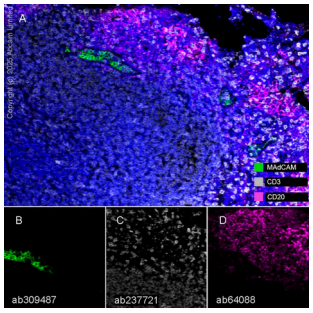
Panel C: ant-CD3 staining B lymphocytes in rat lymph node.

Panel D: ant-CD20 staining B lymphocytes in rat lymph node.

Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab309487, ab237721 and ab64088 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 [CAL57] (ab237721)

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

Immunohistochemistry analysis of (Formalin/PFA-fixed paraffin-embedded sections) Mouse lymph node tissue labelling MAdCAM1 with ab309487 at 1:100 dilution (B), CD3 epsilon with ab237721at 1:2000 dilution (C) and CD20 with ab64088 at 1:100 dilution (D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-MAdCAM (green; Opal™520), anti-CD3 (gray; Opal™690) and anti-CD20 (magenta; Opal™570) on mouse lymph node.

Panel B: anti-MAdCAM staining high endothelial venules in mouse lymph node.

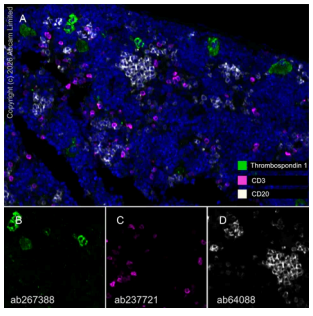
Panel C: ant-CD3 staining B lymphocytes in mouse lymph node.

Panel D: ant-CD20 staining B lymphocytes in mouse lymph node.

Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab309487, ab237721 and ab64088 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 [CAL57] (ab237721)

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 Thrombospondin 1 with ab267388 at a 1/5000 dilution, ab237721 anti-CD3 used at 1/2000 dilution and ab64088 anti-CD20 used at a 1/100 dilution.
Panel A: merged staining of anti-Thrombospondin 1 (green; Opal™520), anti-CD3 (magenta; Opal™690) and anti-CD20 (gray; Opal™570) on mouse spleen.

Panel B: anti-Thrombospondin 1 staining megakaryocytes or platelet in mouse spleen.

Panel C: anti-CD3 staining T lymphocytes in mouse spleen.

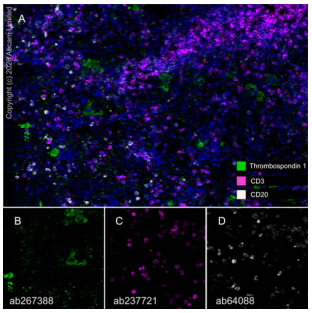
Panel D: anti-CD20 staining B lymphocytes in mouse spleen.

Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab267388, ab237721 and ab64088 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.

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 [CAL57] (ab237721)

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 staining Thrombospondin 1 with ab267388 at a 1/5000 dilution, ab237721 anti-CD3 used at 1/2000 dilution and ab64088 anti-CD20 used at a 1/100 dilution.
Panel A: merged staining of anti-Thrombospondin 1 (green; Opal™520), anti-CD3 (magenta; Opal™690) and anti-CD20 (gray; Opal™570) on rat spleen.

Panel B: anti-Thrombospondin 1 staining megakaryocytes or platelet in rat spleen.

Panel C: anti-CD3 staining T lymphocytes in rat spleen.

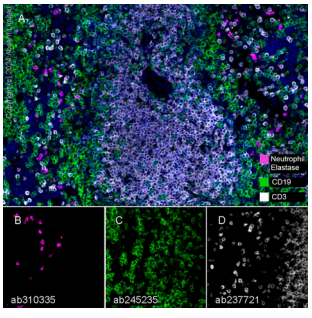
Panel D: anti-CD20 staining B lymphocytes in rat spleen.

Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab267388, ab237721 and ab64088 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.

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 [CAL57] (ab237721)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Mouse spleen tissue staining Neutrophil Elastase with ab310335 at a 1/500 (1.076 µg/ml) dilution, CD19 with ab245235 at a 1/1000 (0.444 µg/ml) dilution and CD3 with ab237721 at a 1/2000 (0.264 µg/ml) dilution followed by a ready to use secondary antibody Opal Polymer HRP Ms + Rb.

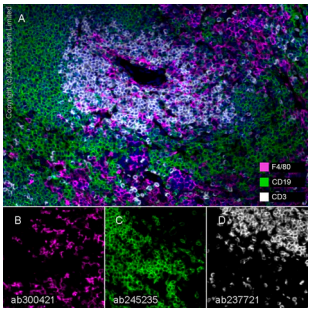
Panel A: merged staining of anti-Neutrophil Elastase (magenta; Opal™690), anti-CD19 (green; Opal™520) and anti-CD3 (gray; Opal™690) on mouse spleen.Panel B: anti-Neutrophil Elastase staining neutrophils in mouse spleen.
Panel C: anti-CD19 staining B lymphocytes in mouse spleen.
Panel D: anti-CD3 staining 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 ab310335, ab245235 and ab237721 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.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

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Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

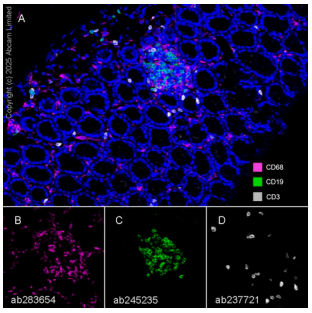
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Mouse spleen staining of F4/80 with ab300421 at a 1/5000 (0.1 µg/ml) dilution, CD19 with ab245235 at 1/1000 (0.444 µg/ml) dilution and CD3 with ab237721 at 1/2000 (0.264 µg/ml) dilution.

Panel A: merged staining of anti-F4/80 (magenta; Opal™690), anti-CD19 (green; Opal™520) and anti-CD3 (gray; Opal™570) on mouse spleen.
Panel B: anti-F4/80 staining macrophages 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 labelled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab300421, ab245235 and ab237721 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.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0 epitope retrieval solution2) for 20 mins.



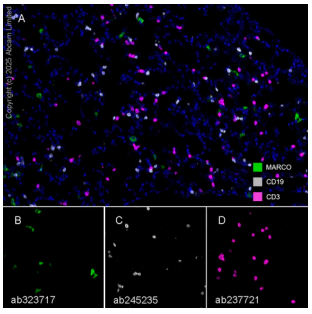
Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse colon tissue staining CD68 with ab283654 at a 1/100 dilution, ab245235 anti-CD19 used at 1/1000 dilution and ab237721 anti-CD3 used at a 1/2000 dilution. Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD68 (magenta; Opal™520), anti-CD19 (green; Opal™690) and anti-CD3 (grey; Opal™570) on mouse colon.
Panel B: anti-CD68 staining macrophages in mouse colon.
Panel C: anti-CD19 staining B lymphocytes in mouse colon.
Panel D: anti-CD3 staining T lymphocytes in mouse colon.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab283654, ab245235 and ab237721 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 [CAL57] (ab237721)

CD3 epsilon Multiplex immunohistochemistry staining of mouse lung tissue using rabbit Anti-CD3 epsilon antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse lung tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab245235 anti-CD19 used at 1:1000 (0.22 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

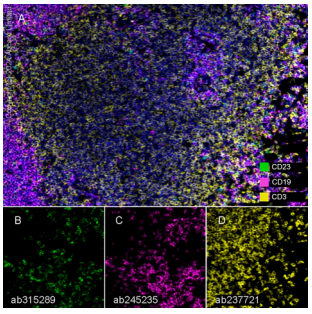
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD19 (gray; Opal™690) and anti-CD3 (magenta; Opal™570) on mouse lung.
Panel B: anti-MARCO staining macrophages in mouse lung.
Panel C: ant-CD19 staining B lymphocytes in mouse lung.
Panel D: ant-CD3 staining T lymphocytes in mouse lung.
Nuclear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

The section was incubated in three rounds of staining: in the order of ab323717, ab245235 and ab237721 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.



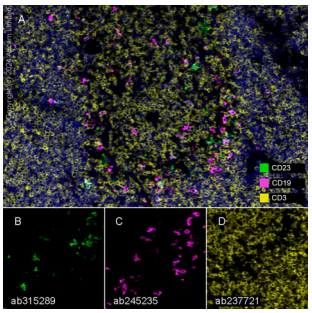
Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse lymph node tissue staining CD23 with ab315289 at a 1/2000 dilution (0.253 µg/ml), CD19 with ab245235 at 1/1000 dilution (0.444 µg/ml), and CD3 with ab237721 at 1/8000 dilution (0.07 µg/ml), followed by a ready to use secondary antibody Opal Polymer HRP Ms + Rb.

Panel A: merged staining of anti-CD23 (green; Opal™520), anti-CD19 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on mouse lymph node.
Panel B: anti-CD23 staining B lymphocytes in mouse lymph node.
Panel C: anti-CD19 staining B lymphocytes in mouse lymph node.
Panel D: anti-CD3 staining T lymphocytes in mouse lymph node.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab315289, ab245235 and ab237721 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.



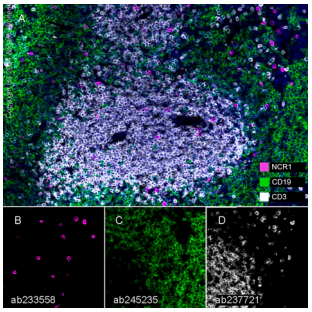
Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse thymus tissue staining CD23 with ab315289 at a 1/2000 dilution (0.253 µg/ml), CD19 with ab245235 at 1/1000 dilution (0.444 µg/ml), and CD3 with ab237721 at 1/8000 dilution (0.07 µg/ml), followed by a ready to use secondary antibody Opal Polymer HRP Ms + Rb.

Panel A: merged staining of anti-CD23 (green; Opal™520), anti-CD19 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on mouse thymus.
Panel B: anti-CD23 staining B lymphocytes in mouse thymus.
Panel C: anti-CD19 staining B lymphocytes in mouse thymus.
Panel D: anti-CD3 staining T lymphocytes in mouse thymus.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab315289, ab245235 and ab237721 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.



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

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

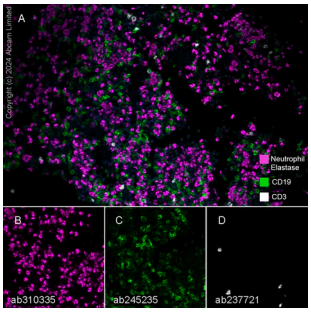
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Mouse spleen staining of NCR1 with ab233558 at a 1/500 (1.062 µg/ml) dilution, CD19 with ab245235 at 1/1000 (0.444 µg/ml) dilution and CD3 with ab237721 at 1/2000 (0.264 µg/ml) dilution.

Panel A: merged staining of anti-NCR1 (magenta; Opal™690), anti-CD19 (green; Opal™520) and anti-CD3 (gray; Opal™570) on mouse spleen.
Panel B: Anti-NCR1 staining natural killer cells 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 labelled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab233558, ab245235 and ab237721 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.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0 epitope retrieval solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL57] (ab237721)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Mouse bone marrow tissue staining Neutrophil Elastase with ab310335 at a 1/500 (1.076 µg/ml) dilution, CD19 with ab245235 at a 1/1000 (0.444 µg/ml) dilution and CD3 with ab237721 at a 1/2000 (0.264 µg/ml) dilution followed by a ready to use secondary antibody Opal Polymer HRP Ms + Rb.

Panel A: merged staining of anti-Neutrophil Elastase (magenta; Opal™690), anti-CD19 (green; Opal™520) and anti-CD3 (gray; Opal™690) on mouse spleen.Panel B: anti-Neutrophil Elastase staining neutrophils 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 ab310335, ab245235 and ab237721 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.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.