abcam

ab236434

Anti-CD20 antibody [SP32] - BSA and Azide free

Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434) is a rabbit recombinant monoclonal antibody provided in a PBS only buffer for easy conjugation. Suitable for **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF, mIHC** in **Human, Mouse, Rat**.

- BSA, sodium azide, and glycerol-free for easy conjugation
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | Advanced Validation

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	SP32
Purification technique	Affinity purification Protein A/G
Concentration	1.009 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purified from TCS by protein A/G.

Reactivity data

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat, Cow, Dog, Cat, Sheep
Dilution info	Use at an assay dependent concentration.
Notes	-

mIHC

Tested

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

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

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

Expected

Species	Cow, Dog, Cat, Sheep
Dilution info	Use at an assay dependent concentration.

Notes	-

IHC-P

Tested

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

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

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

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

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

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

Species	Sheep
Dilution info	-
Notes	

	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

WB

Expected

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

Predicted

Species	Cow, Dog, Cat, Sheep
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat, Cow, Dog, Cat, Sheep
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-

Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Species	Human
Dilution info	-
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat, Cow, Dog, Cat, Sheep
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information MS4A1

Function	B-lymphocyte-specific membrane protein that plays a role in the regulation of cellular calcium influx necessary for the development, differentiation, and activation of B-lymphocytes (PubMed:12920111, PubMed:3925015, PubMed:7684739). Functions as a store-operated calcium (SOC) channel component promoting calcium influx after activation by the B-cell receptor/BCR (PubMed:12920111, PubMed:18474602, PubMed:7684739).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434) 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), Immunocytochemistry/immunofluorescence (ICC/IF), Multiplex IHC (mIHC) in Human, Mouse, Rat samples.

Collaboration
Anti-CD20 [SP32] - BSA and Azide free (ab236434) is a clone from the portfolio of Spring Bioscience (Roche) SP clones which have been optimised for immunohistochemistry (IHC).

Other related products
We have a range of other formats of antibody clone [SP32] also available for your convenience: ab64088, Carrier free - ab236434, Oligonucleotide - ab279356, ab279467, PE - ab305647, APC - ab305648, HRP - ab305649, Alexa Fluor® 488 - ab307132, Alkaline Phosphatase - ab308806, Alexa Fluor® 594 - ab310536, Alexa Fluor® 555 - ab312067, Alexa Fluor® 568 - ab312544, Alexa Fluor® 750 - ab321688

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.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

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

Activity summary	CD20 also known as MS4A1 or L26 is a membrane-spanning 4-domains subfamily A member 1 protein. It is a non-glycosylated phosphoprotein with a molecular mass of approximately 33-37 kDa. Expressed extensively on the surface of B-cells CD20 plays an important role in B-cell activation and regulation. While CD20 is absent on early pro-B cells and plasma cells its presence increases as B-cells mature. Anti-CD20 antibodies such as 2H7 are commonly used in research and treatment to deplete B-cells due to the target's consistent expression in most stages of B-cell development.
Biological function summary	CD20 contributes significantly to calcium ion transport which is essential for the activation process of B-cells. Although CD20 does not belong to a larger protein complex its role centers around forming a calcium channel that allows a sustained

	influx of calcium into the B-cells. This influx is important for the signaling pathways that govern B-cell activation proliferation and differentiation impacting the immune response efficacy.
Pathways	CD20 is closely involved in the B-cell receptor (BCR) signaling pathway and the Fc gamma R-mediated phagocytosis pathway. These pathways play vital roles in adaptive immunity and immune response modulation. CD20's interaction with proteins like PI3K during BCR signaling enhances receptor-mediated cellular signals subsequently influencing downstream effectors involved in cell growth and survival of B-cells.
Associated diseases and disorders	CD20 is strongly linked to certain blood cancers and autoimmune diseases including non-Hodgkin's lymphoma and rheumatoid arthritis. These conditions often exploit aberrant B-cell activity or count. CD20's expression on malignant B-cells in non-Hodgkin's lymphoma makes it an effective target for monoclonal antibody therapies such as rituximab an anti-CD20 antibody. Additionally CD20-targeted therapies can help deplete pathogenic B-cells in rheumatoid arthritis highlighting the protein's relevance in disease treatment.

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

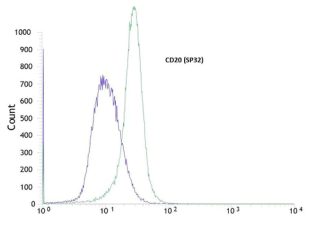
We do not recommend this combination. It is not covered by our product promise.

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.

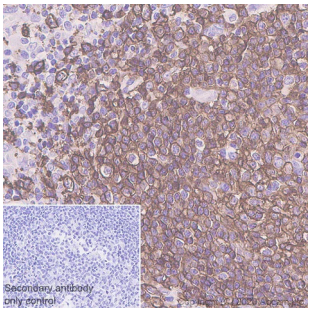
17 product images



Flow Cytometry (Intracellular) - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Flow Cytometry (Intracellular) staining using rabbit Anti-CD20 antibody

Flow cytometric analysis of rabbit anti-CD20 (SP32) antibody ab64088(1/100) in Jurkat cells (green) compared to negative control of rabbit IgG (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab64088).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human tonsil using rabbit Anti-CD20 antibody

This data was developed using ab64088, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling CD20 with ab64088 at 1/100 dilution.

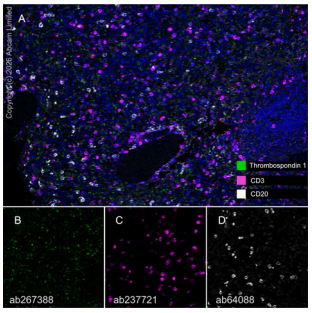
Positive staining on human tonsil.

The section was incubated with ab64088 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 (pH 6.0, Epitope Retrieval Solution1) for 10 mins.



Multiplex immunohistochemistry - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Multiplex immunohistochemistry staining of Human spleen using rabbit Anti-CD20 antibody

This data was developed using ab64088, the same antibody clone in a different buffer formulation. 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.

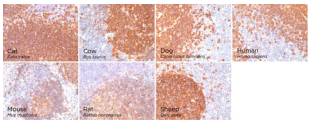
Nuclear DNA was labeled with DAPI (shown in blue).

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.

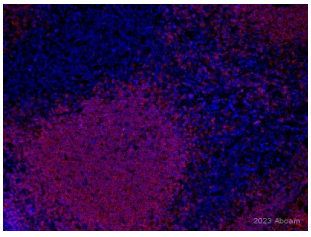


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD20 antibody

This data was developed using ab64088, the same antibody clone in a different buffer formulation The BOND™ Polymer Refine Detection System on the BOND RX Automated Stainer (DS9800, Leica Biosystems, Wetzlar, Germany) was used for immunohistochemical labelling of formalin-fixed, paraffin-embedded (FFPE) tissues. The protocol includes a peroxide block, post-primary reagent, polymer detection, DAB chromogen, and haematoxylin counterstain, all automated to minimize variability. Antigen retrieval was performed by heat-induced epitope retrieval (HIER) for 20 minutes in Tris-EDTA buffer (BOND Epitope Retrieval Solution 2, AR9640, Leica Biosystems, Wetzlar, Germany). For mouse and rabbit antibodies, the sequence comprised peroxide block (10 min), primary antibody incubation (30 min), post-primary (10 min), polymer (10 min), DAB (10 min), and haematoxylin (8 min). For rat antibodies, an anti-rat step (1:300 dilution, Vector Laboratories, California, USA; 20 min) was included prior to polymer application (15 min).

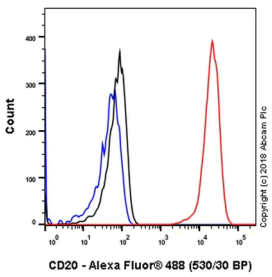
For mouse tissues, antigen retrieval was performed using high pH buffers (Dako, Agilent), followed by blocking of endogenous peroxidase activity with 3% hydrogen peroxide. Slides were then incubated with the primary antibody. Antigen/antibody complexes detection was carried out using a horseradish peroxidase-conjugated visualization system (Novolink Polymer, Leica). Staining was developed with 3,3′-diaminobenzidine (EnVision FLEX DAB Enhancer, Dako, Agilent), and nuclei were counterstained with hematoxylin (FLEX Hematoxylin, SM806, Dako, Agilent). Images were acquired using an Axiocam CCD camera (Zeiss) and processed with ZEN 2.1 software and Photoshop 9.0.



This image is courtesy of a customer review submitted by Natalie Papazian

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

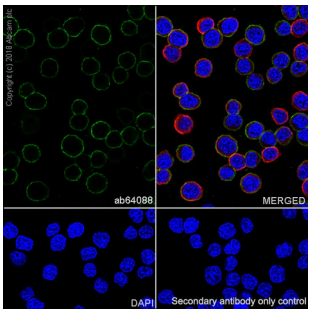
Immunohistochemistry analysis of paraformaldehyde fixed Human tonsil tissue staining CD20 with ab236434 at 1/400 and Alexa Fluor 555 donkey anti rabbit secondary. The tissue was incubated with the primary antibody for 16 hours at 4 degrees with PBS + 2% FCS. 5% normal human serum was used for blocking for 1 hour at room temperature.



Flow Cytometry (Intracellular) - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Flow Cytometry (Intracellular) staining using rabbit Anti-CD20 antibody

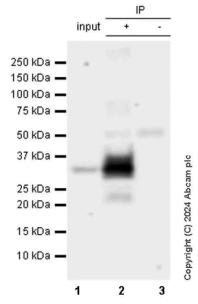
Flow cytometry analysis of Ramos (human Burkitt's lymphoma) labeling CD20 with purified ab64088 at 1/40 dilution (7.78 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody. Isotypecontrol - Rabbit monoclonal IgG (ab172730) (black). Unlabeled control - Unlabelled cells (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab64088).



Immunocytochemistry/ Immunofluorescence - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD20 antibody

Immunocytochemistry/ Immunofluorescence analysis of Ramos (human Burkitt's lymphoma B lymphocyte) cells labeling CD20 with purified ab64088 at 1/100 (3.1 µg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab64088).



Immunoprecipitation - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

ab64088 at 1/30 dilution immunoprecipitating CD20 in Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate. Western blot was performed from the immunoprecipitate using ab64088 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution. Lane 1: Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate 10 µg Lane 2: ab64088 IP in Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab64088 in Raji whole cell lysate Blocking/Dilution buffer: 5% NFDM/TBST. Exposure time: 8 seconds. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab64088).
Lane 2:
Immunoprecipitation - Anti-CD20 antibody [SP32] (ab64088) at 1/30 dilution

Lane 2:
Immunoprecipitation - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

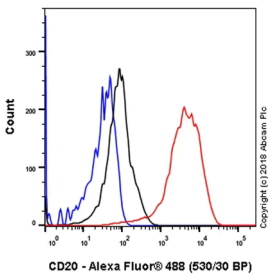
All lanes:
HeLa (human cervix adenocarcinoma epithelial cell) treated with 500 µM CoCl2 for 24 hours whole cell lysate at 10 µg

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 25 kDa, 33 kDa

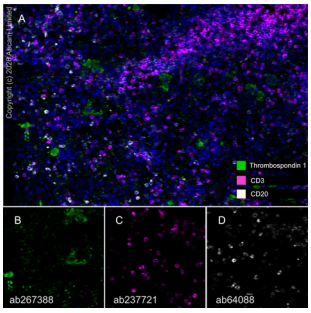
Exposure time: 8s



Flow Cytometry (Intracellular) - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Flow Cytometry (Intracellular) staining using rabbit Anti-CD20 antibody

Flow cytometry analysis of WEHI-231 (mouse lymphoblast B cell lymphoma) labeling CD20 with purified ab64088 at 1/40 dilution (7.78 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody. Isotypecontrol - Rabbit monoclonal IgG (ab172730) (black). Unlableled control - Unlabelled cells (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab64088).



Multiplex immunohistochemistry - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Multiplex immunohistochemistry staining of Rat spleen using rabbit Anti-CD20 antibody

This data was developed using ab64088, the same antibody clone in a different buffer formulation. 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.

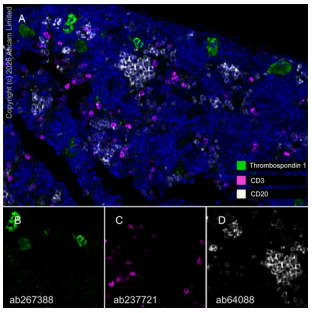
Panel D: anti-CD20 staining B lymphocytes in rat spleen.

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-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Multiplex immunohistochemistry staining of Mouse spleen using rabbit Anti-CD20 antibody

This data was developed using ab64088, the same antibody clone in a different buffer formulation. 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.

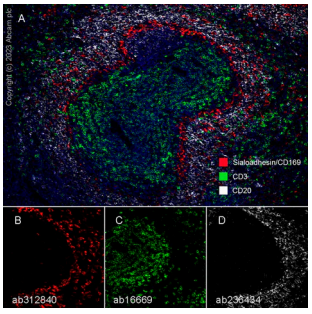
Panel D: anti-CD20 staining B lymphocytes in mouse spleen.

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.

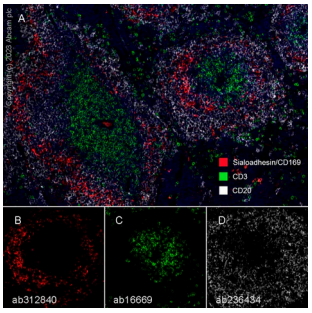
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Multiplex immunohistochemistry staining of Rat spleen using rabbit Anti-CD20 antibody

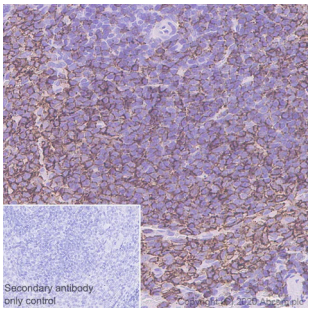
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Rat spleen tissue labeling Sialoadhesin/CD169, CD3 epsilon and CD20 with ab312840 at 1/100 dilution, ab16669 at 1:150 dilution and ab236434 at 1:5000 dilution. Panel A: merged staining of anti-Sialoadhesin/CD169 (red; Opal™690), 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-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Multiplex immunohistochemistry staining of Mouse spleen using rabbit Anti-CD20 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Rat spleen using rabbit Anti-CD20 antibody

This data was developed using ab64088, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling CD20 with ab64088 at 1/100 dilution.

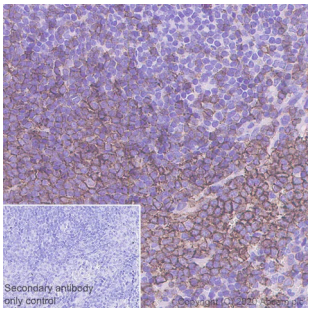
Positive staining on rat spleen.

The section was incubated with ab64088 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 (pH 6.0, Epitope Retrieval Solution1) for 10 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse spleen using rabbit Anti-CD20 antibody

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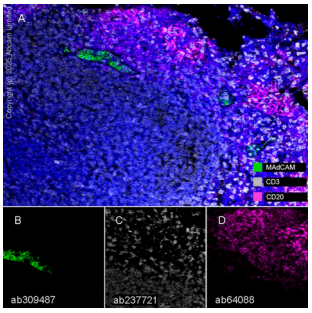
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Multiplex immunohistochemistry - Anti-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Multiplex immunohistochemistry staining of Mouse lymph node using rabbit Anti-CD20 antibody

This data was developed using ab64088, the same antibody clone in a different buffer formulation.

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.

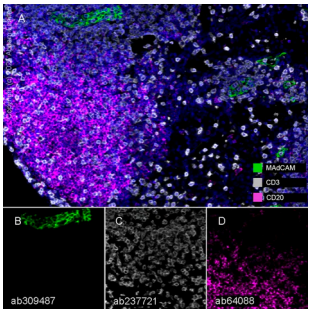
Panel C: ant-CD3 staining B lymphocytes in mouse lymph node.

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-CD20 antibody [SP32] - BSA and Azide free (ab236434)

CD20 Multiplex immunohistochemistry staining of Rat lymph node using rabbit Anti-CD20 antibody

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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.

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