abcam

ab166865

Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP459Y
Purification technique	Affinity purification Protein A
Specificity	For optimal performance in IHC, the primary antibody should be incubated overnight at 4 .
Concentration	1.011 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat, Monkey
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat, Monkey
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	For optimal performance in IHC, the primary antibody should be incubated overnight at 4 . This product did not work well on the Leica BOND system under our internal testing conditions.

Not recommended

Species	Mouse, Rat, Monkey
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat, Monkey
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat, Monkey
Dilution info	-
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

ab166865 is the carrier-free version of ab78237.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

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.

What does low endotoxin mean?
Our low endotoxin, azide-free formats have low endotoxin level (1 EU/mg, determined by the TAL assay) and are free

from azide, to achieve consistent experimental results in functional assays.

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.

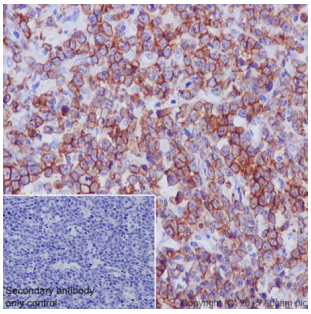
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

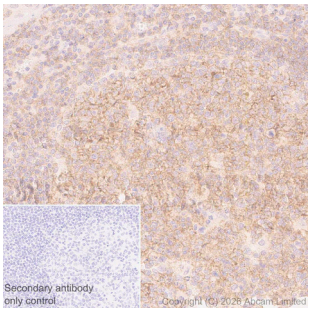
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8 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

This IHC data was generated using the same anti-CD20 antibody clone, EP459Y, in a different buffer formulation (cat# ab78237).
Immunohistochemical staining of paraffin embedded human B cell lymphoma with purified ab78237 at a working dilution of 1/50. The secondary antibody used is ab97051, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

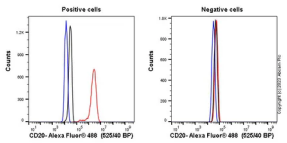


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab78237).
Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil tissue labelling CD20 with ab78237 at a 1/100 dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymoer) ab214880. The section was incubated with ab78237 overnight at 4°C.

The section was counterstained with Hematoxylin. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0)



Flow Cytometry (Intracellular) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

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

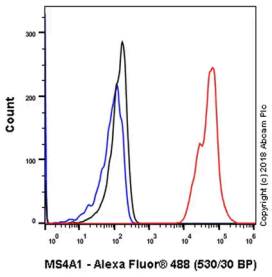
This data was developed using the same antibody clone in a different buffer formulation containing PBS BSA glycerol and sodium azide (ab78237).
Flow cytometry overlay histogram showing left Ramos positive cells and right negative HEK293 stained with ab78237 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab78237) (1x 106 in 100µl at 0.2µg/ml (1/2500)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in Ramos Fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



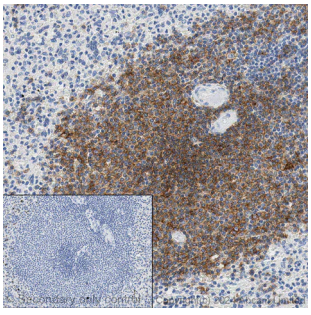
Flow Cytometry (Intracellular) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

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

Intracellular Flow Cytometry analysis of Ramos (Human Burkitt's lymphoma B lymphocyte) labeling CD20 with purified ab78237 at 1/200 dilution (Red). Goat anti rabbit IgG (Alexa Fluor®488, ab150081) at 1/2000 dilution was used as secondary antibody. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol.
Isotype control: Rabbit monoclonal IgG (ab172730) (Black)

Unlabelled cells: (Blue)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab78237).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

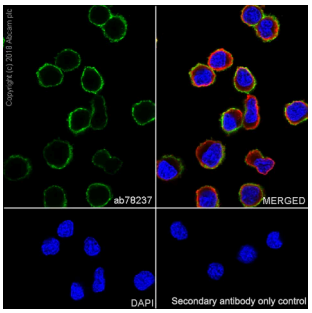
CD20 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human spleen using rabbit Anti-CD20 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab78237).
Immunohistochemical analysis of formalin fixed paraffin embedded human spleen labelling CD20 with ab78237 at a concentration of 1 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit.

Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH 8.5 for 32mins.

ab78237 anti-CD20 [EP459Y] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

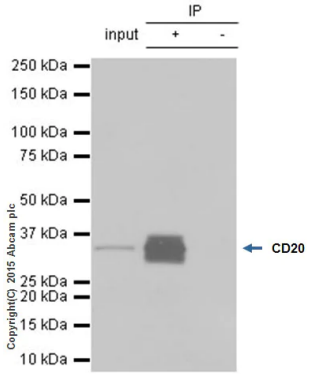
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual).



Immunocytochemistry/ Immunofluorescence - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

CD20 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD20 antibody

Immunocytochemistry/ Immunofluorescence analysis of Ramos (human Burkitt's lymphoma B lymphocyte) labeling CD20 with purified ab78237 at 1/10 dilution. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/1000 was used as the secondary antibody. Cells were counterstained with ab195889, Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200. PBS instead of the primary antibody was used as negative control. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Nuclei were stained with DAPI (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab78237).

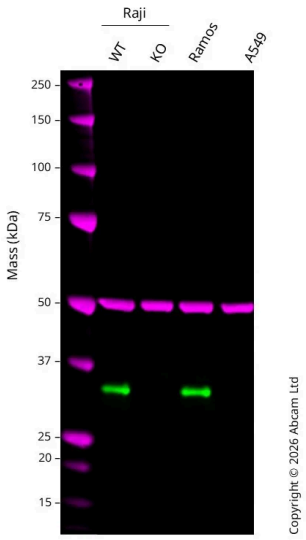


Immunoprecipitation - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

ab78237 (purified) at 1/20 immunoprecipitating CD20 in 10 µg Ramos cell lysate (Lanes 1 and 2, observed at 33 kDa). Lane 3 - Rabbit monoclonal IgG (ab172730). For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution. Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab78237).

All lanes:
Immunoprecipitation - Anti-CD20 antibody [EP459Y] (ab78237)

Predicted band size: 33 kDa



Western blot - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

CD20 Western blot staining using rabbit Anti-CD20 antibody

This data was developed using ab78237, the same antibody clone in a different buffer formulation. Blocking and diluting buffer and concentration: 3% NFDM/TBST.

Western blot: Anti-CD20 antibody [EP459Y] ab78237 staining at 1/1000 dilution, shown in green; Mouse anti alpha Tubulin ab7291 loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 33 kDa in Wild-type Raji cell lysates with no signal observed at this size in MS4A1 knockout Raji ab273871 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.

All lanes:
Western blot - Anti-CD20 antibody [EP459Y] (ab78237) at 1/1000 dilution

Lane 1:
Wild-type Raji (human B-lymphocyte cell line) cell lysates

Lane 2:
CD20 knockout Raji (human B-lymphocyte cell line) cell lysate

Lane 2:
Western blot - Human MS4A1 (CD20) knockout Raji cell line (ab273871)

Lane 3:
Ramos (human B lymphocyte cell line) cell lysate

Lane 4:
A549 (human alveolar basal epithelial cell line) cell lysate

Secondary

All lanes:
Goat anti-Rabbit 800CW at 1/20000 dilution

Observed band size: 33 kDa, 50 kDa

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