abcam

ab279299

Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric)

Recombinant | KO Validated | 20ul selling size

Key facts

Isotype	IgG2a
Host species	Mouse
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP459Y
Purification technique	Affinity purification Protein A
Concentration	0.813 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC

Tested

Species	Human
Dilution info	0.2-1 µg/mL
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	0.2 µg/mL
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 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

This mouse monoclonal chimeric antibody has been engineered from a RabMAb parent antibody (ab78237). By necessity, some rabbit sequence is retained as part of the variable domain. When multiplexing with other rabbit-derived antibodies, using cross absorbed Fc-reactive secondary antibodies are recommended.

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

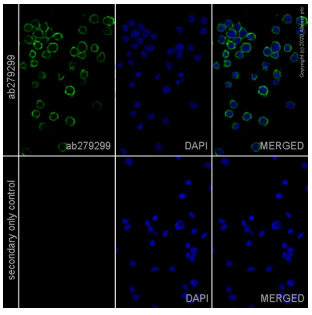
We do not recommend this combination. It is not covered by our product promise.

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.

6 product images



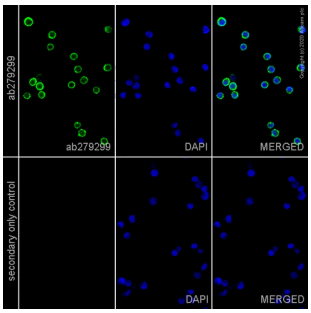
Immunocytochemistry - Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (ab279299)

CD20 Immunocytochemistry staining using mouse Anti-CD20 antibody

Immunofluorescence staining of CD20 using ab279299 in Ramos (human Burkitt's lymphoma cell line) cells.
The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab279299 at 0.2 µg/ml. Cells were then incubated with ab150117, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and nuclear DNA was labelled with DAPI (shown in blue).

The secondary only control (bottom row) was not incubated with ab279299 but otherwise processed the same.

Images were acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



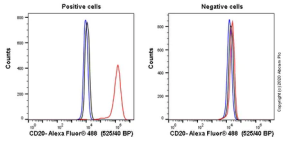
Immunocytochemistry - Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (ab279299)

CD20 Immunocytochemistry staining using mouse Anti-CD20 antibody

Immunofluorescence staining of CD20 using ab279299 in Ramos (human Burkitt's lymphoma cell line) cells.
The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab279299 at 0.2 µg/ml. Cells were then incubated with ab150117, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and nuclear DNA was labelled with DAPI (shown in blue).

The secondary only control (bottom row) was not incubated with ab279299 but otherwise processed the same.

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Flow Cytometry (Intracellular) - Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (ab279299)

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

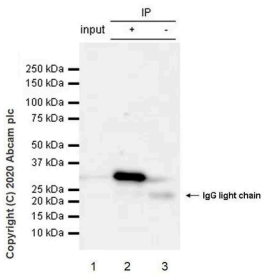
Flow cytometry overlay histogram showing Ramos (human Burkitt's lymphoma cell line) positive cells (left panel) and negative HEK293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells (right panel) stained with ab279299 (red line).
The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab279299) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 22°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (ab150177) was used at 1/2000 for 30 min at 22°C.

Isotype control antibody (black line) was mouse IgG2a kappa (ab18413) 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 cells fixed with 80% methanol (5 min) / permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Immunoprecipitation - Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (ab279299)

CD20 was immunoprecipitated from 0.35 mg Ramos (human Burkitt's lymphoma B lymphocyte), whole cell lysate 10 µg with ab279299 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab279299 at 1/1000 dilution. mouse IgG for IP (HRP) (ab131368) was used at 1/5000 dilution.
Lane 1: Ramos whole cell lysate 10µg.

Lane 2: ab279299 IP in Ramos whole cell lysate.

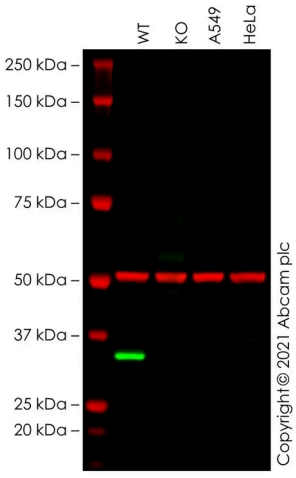
Lane 3: Mouse monoclonal IgG2a (ab18413) instead of ab279299 in Ramos whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 5.5 seconds.

All lanes:
Immunoprecipitation - Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (ab279299)

Predicted band size: 33 kDa



Western blot - Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (ab279299)

CD20 Western blot staining using mouse Anti-CD20 antibody

False colour image of Western blot: Anti-CD20 antibody [EP459Y] – Mouse IgG2a (Chimeric) staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (ab52866) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab279299 was shown to bind specifically to CD20. A band was observed at 33 kDa in wild-type Raji cell lysates with no signal observed at this size in MS4A1 knockout cell line ab273871 (knockout cell lysate ab273825). To generate this image, wild-type and MS4A1 knockout Raji cell lysates were analysed. First, 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.

All lanes:
Western blot - Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (ab279299) at 1/1000 dilution

Lane 1:
Wild-type Raji cell lysate at 20 µg

Lane 2:
Western blot - Human MS4A1 knockout Raji cell lysate (ab273825) at 20 µg

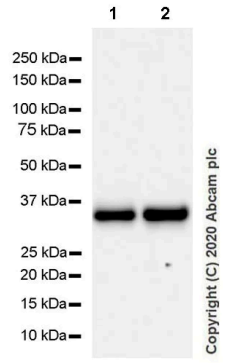
Lane 3:
A549 cell lysate at 20 µg

Lane 4:
HeLa cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 21 kDa, 33 kDa

Observed band size: 33 kDa



Western blot - Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (ab279299)

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (ab279299) at 1/1000 dilution

Lane 1:
Raji (human Burkitt's lymphoma B lymphocyte), whole cell lysate at 20 µg

Lane 2:
Ramos (human Burkitt's lymphoma B lymphocyte), whole cell lysate at 20 µg

Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Predicted band size: 33 kDa

Exposure time: 10s

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