abcam

Product datasheet

Anti-CD20 antibody [EP459Y] - Mouse IgG1 (Chimeric) ab279298

KO VALIDATED Recombinant

6 Images

Overview

Product name	Anti-CD20 antibody [EP459Y] - Mouse IgG1 (Chimeric)
Description	Mouse monoclonal [EP459Y] to CD20 - Mouse IgG1
Host species	Mouse
Tested applications	**Suitable for:** WB, IP, Flow Cyt (Intra), ICC
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Raji and Ramos whole cell lysate. ICC: Ramos cells. Flow Cyt (intra): Ramos cells. IP: Ramos whole cell lysate.
General 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.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP459Y
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab279298 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000.
IP		1/30.
Flow Cyt (Intra)		Use a concentration of 0.2 µg/ml.
ICC		Use a concentration of 0.2 - 1 µg/ml.

Target

Function This protein may be involved in the regulation of B-cell activation and proliferation.

Tissue specificity Expressed on B-cells.

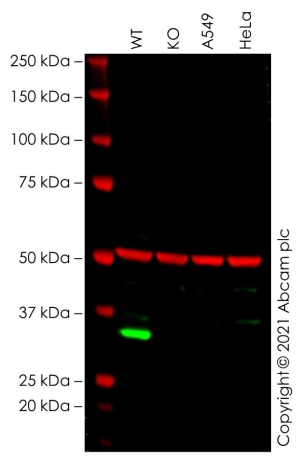
Involvement in disease Defects in MS4A1 are the cause of immunodeficiency common variable type 5 (CVID5) [MIM:613495]; also called antibody deficiency due to CD20 defect. CVID5 is a primary immunodeficiency characterized by antibody deficiency, hypogammaglobulinemia, recurrent bacterial infections and an inability to mount an antibody response to antigen. The defect results from a failure of B-cell differentiation and impaired secretion of immunoglobulins; the numbers of circulating B cells is usually in the normal range, but can be low.

Sequence similarities Belongs to the MS4A family.

Post-translational modifications Phosphorylated. Might be functionally regulated by protein kinase(s).

Cellular localization Membrane.

Images



Western blot - Anti-CD20 antibody [EP459Y] - Mouse IgG1 (Chimeric) (ab279298)

All lanes : Anti-CD20 antibody [EP459Y] - Mouse IgG1 (Chimeric) (ab279298) at 1/1000 dilution

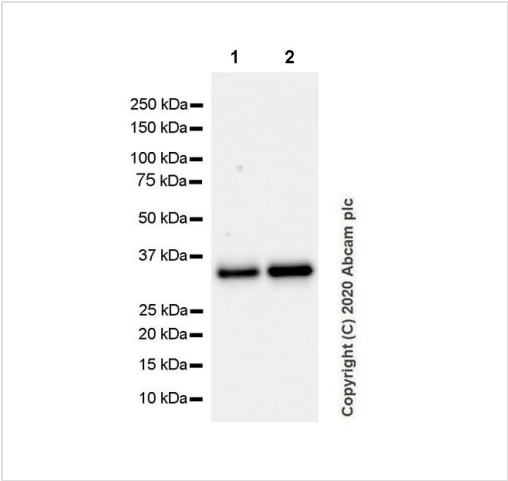
Lane 1 : Wild-type Raji cell lysate
Lane 2 : MS4A1 knockout Raji cell lysate
Lane 3 : A549 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 33 kDa

False colour image of Western blot: Anti-CD20 antibody [EP459Y] – Mouse IgG1 (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, ab279298 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 **ab263259**). 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.



Western blot - Anti-CD20 antibody [EP459Y] - Mouse IgG1 (Chimeric) (ab279298)

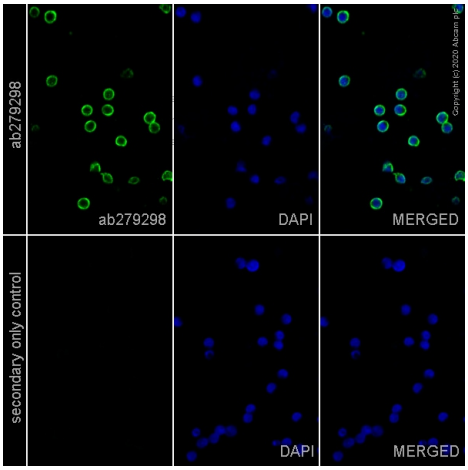
All lanes : Anti-CD20 antibody [EP459Y] - Mouse IgG1 (Chimeric) (ab279298) at 1/1000 dilution

Lane 1 : Raji (human Burkitt's lymphoma B lymphocyte), whole cell lysate
Lane 2 : Ramos (human Burkitt's lymphoma B lymphocyte), whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Blocking/Dilution buffer: 5% NFDM/TBST.



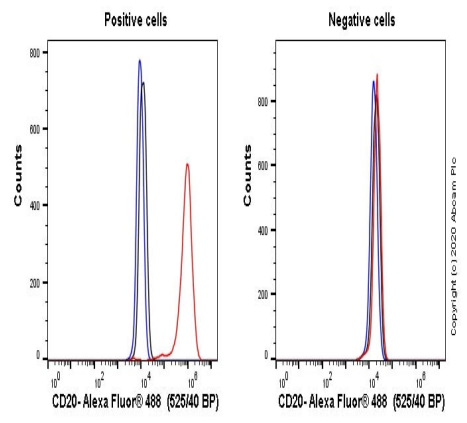
Immunocytochemistry - Anti-CD20 antibody [EP459Y] - Mouse IgG1 (Chimeric) (ab279298)

Immunofluorescence staining of CD20 using ab279298 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 ab279298 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 ab279298 but otherwise processed the same.

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



Flow Cytometry (Intracellular) - Anti-CD20 antibody [EP459Y] - Mouse IgG1 (Chimeric) (ab279298)

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 ab279298 (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 (ab279298) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 22°C.

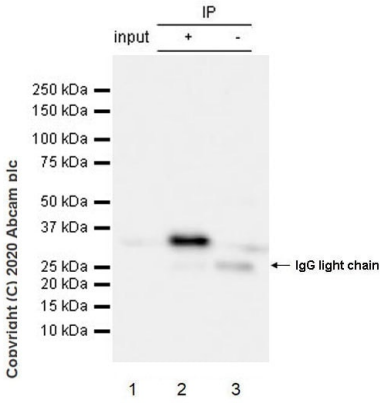
The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150177**) was used at 1/2000 for 30 min at 22°C.

Isotype control antibody (black line) was mouse IgG1 kappa (**ab170190**) 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 IgG1 (Chimeric) (ab279298)

CD20 was immunoprecipitated from 0.35 mg Ramos (human Burkitt's lymphoma B lymphocyte), whole cell lysate 10 µg with ab279298 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab279298 at 1/1000 dilution. Mouse IgG for IP (HRP) (**ab131368**) was used at 1/5000 dilution.

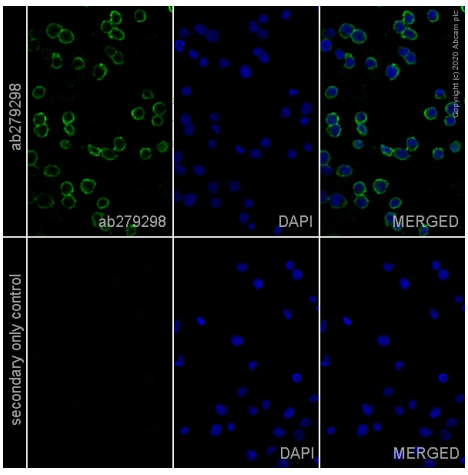
Lane 1: Ramos whole cell lysate 10µg.

Lane 2: ab279298 IP in Ramos whole cell lysate.

Lane 3: Mouse monoclonal IgG1 (**ab18443**) instead of ab279298 in Ramos whole cell lysate.

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

Exposure time: 5.5 seconds.



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