abcam

Product datasheet

Anti-CD20 antibody [EP459Y] - Rat IgG2a (Chimeric) ab279300

KO 評価済 リコンビナント

1 References 画像数 6

製品の概要

製品名	Anti-CD20 antibody [EP459Y] - Rat IgG2a (Chimeric)
製品の詳細	Rat monoclonal [EP459Y] to CD20 - Rat IgG2a
由来種	Rat
アプリケーション	**適用あり:** IP, Flow Cyt (Intra), WB, ICC
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Raji and Ramos whole cell lysate and Wild-type Raji cell lysate ICC: Ramos cells. IP: Ramos whole cell lysate. Flow Cyt (intra): Ramos cells.
特記事項	This rat 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.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EP459Y
アイソタイプ	IgG2a

アプリケーション

The Abpromise guarantee	**Abpromise保証は、次のテスト済みアプリケーションにおけるab279300の使用に適用されます**

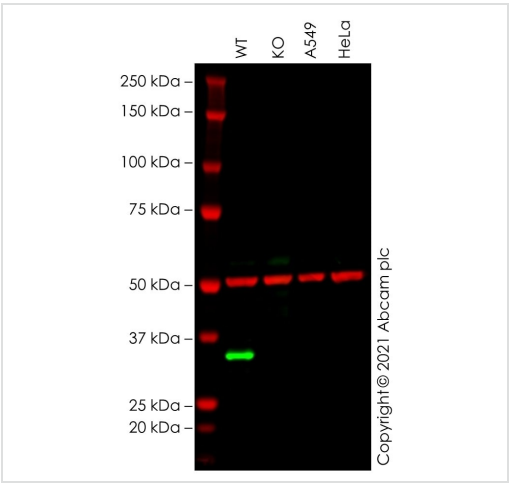
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IP		1/30.
Flow Cyt (Intra)		Use a concentration of 0.2 µg/ml.
WB		1/1000.
ICC		Use a concentration of 0.2 - 1 µg/ml.

ターゲット情報

機能	This protein may be involved in the regulation of B-cell activation and proliferation.
組織特異性	Expressed on B-cells.
関連疾患	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.
配列類似性	Belongs to the MS4A family.
翻訳後修飾	Phosphorylated. Might be functionally regulated by protein kinase(s).
細胞内局在	Membrane.

画像



Western blot - Anti-CD20 antibody [EP459Y] - Rat IgG2a (Chimeric) (ab279300)

All lanes : Anti-CD20 antibody [EP459Y] - Rat IgG2a (Chimeric) (ab279300) at 1/1000 dilution

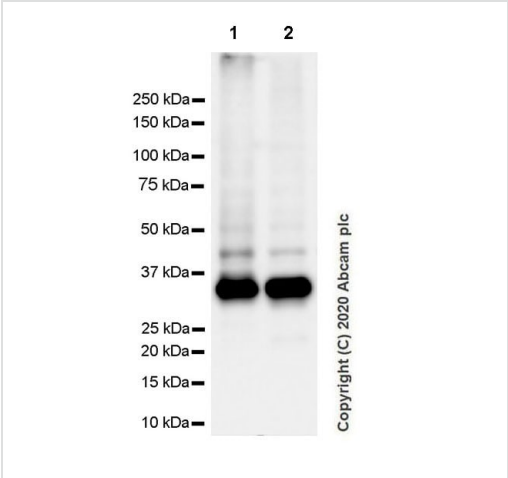
Lane 1 : Wild-type Raji cell lysate
Lane 2 : MS4A1 knockout Raji cell lysate
Lane 3 : A549 cell lysate
Lane 4 : HeLa cell lysate

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] – Rat 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, ab279300 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-Rat IgG H&L (IRDye® 800CW) preabsorbed (**ab253031**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Anti-CD20 antibody [EP459Y] - Rat IgG2a (Chimeric) (ab279300)

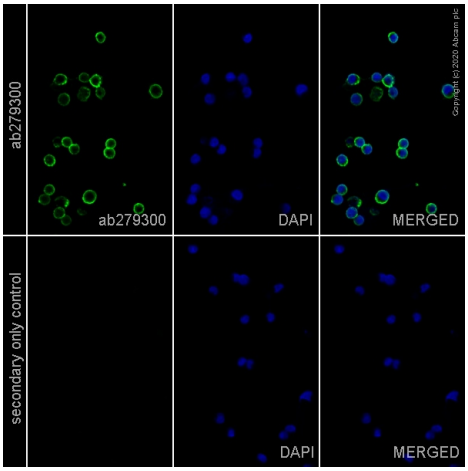
All lanes : Anti-CD20 antibody [EP459Y] - Rat IgG2a (Chimeric) (ab279300) at 1/1000 dilution

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 : Goat Anti-Rat IgG H&L (HRP) (**ab205720**) at 1/5000 dilution

Blocking/Dilution buffer: 5% NFDM/TBST.



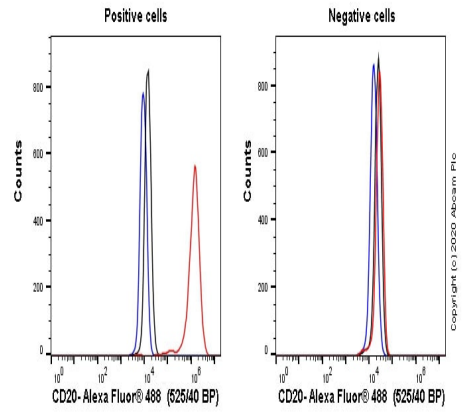
Immunocytochemistry - Anti-CD20 antibody [EP459Y] - Rat IgG2a (Chimeric) (ab279300)

Immunofluorescence staining of CD20 using ab279300 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 ab279300 at 0.2 µg/ml. Cells were then incubated with **ab150165**, Goat Anti-Rat 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 ab279300 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] - Rat IgG2a (Chimeric) (ab279300)

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 ab279300 (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 (ab279300) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 22°C.

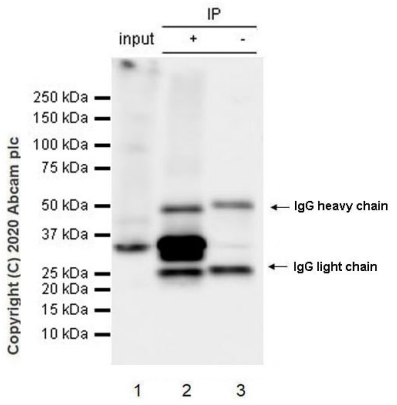
The secondary antibody Goat anti-rat IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150165**) was used at 1/2000 for 30 min at 22°C.

Isotype control antibody (black line) was Rat IgG2a kappa (**ab18450**) 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] - Rat IgG2a (Chimeric) (ab279300)

CD20 was immunoprecipitated from 0.35 mg Ramos (human Burkitt's lymphoma B lymphocyte), whole cell lysate 10 µg with ab279300 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab279300 at 1/1000 dilution. Goat Anti-Rat IgG (H+L), HRP) (**ab205720**) was used at 1/5000 dilution.

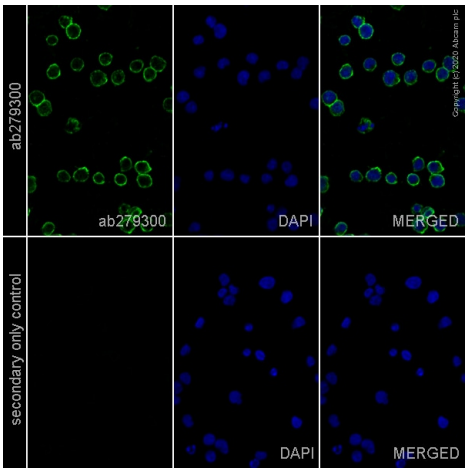
Lane 1: Ramos whole cell lysate 10µg.

Lane 2: ab279300 IP in Ramos whole cell lysate.

Lane 3: Rat monoclonal IgG2a (**ab18450**) instead of ab279300 in Ramos whole cell lysate.

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

Exposure time: 1 second.



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Immunofluorescence staining of CD20 using ab279300 in Ramos (human Burkitt's lymphoma cell line) cells.

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