abcam

Product datasheet

Anti-CXCR5 antibody [EPR23463-30] ab254415

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-CXCR5 antibody [EPR23463-30]
Description	Rabbit monoclonal [EPR23463-30] to CXCR5
Host species	Rabbit
Specificity	We observe only weak staining in human WB. We do not suggest this product for use in IHC with mouse or rat.
Tested applications	**Suitable for:** ICC/IF, WB, Flow Cyt, IHC-P **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Raji, Daudi, Neuro-2a, A20, Mouse spleen, mouse lymph node and rat lymph node, C6 lysates. IHC-P: Human tonsil tissue. Human diffuse large B- lymphoma tissue. Flow Cyt: Human peripheral blood mononuclear and Raji cells. ICC/IF: Daudi cells.
General notes	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR23463-30
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab254415 in the following tested applications.

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

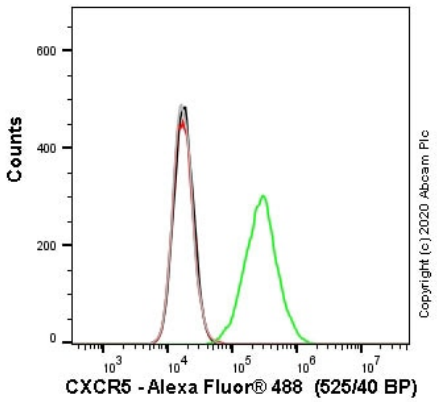
Application	Abreviews	Notes
ICC/IF		Use a concentration of 5 µg/ml.
WB		1/1000. Predicted molecular weight: 42 kDa. We observe only weak staining in human WB.
Flow Cyt	★★★☆☆ (1)	1/500.
IHC-P		1/5000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. We do not suggest this product for use in IHC with mouse or rat.

Application notes Is unsuitable for IP.

Target

Function	Cytokine receptor that binds to B-lymphocyte chemoattractant (BLC). Involved in B-cell migration into B-cell follicles of spleen and Peyer patches but not into those of mesenteric or peripheral lymph nodes. May have a regulatory function in Burkitt lymphoma (BL) lymphomagenesis and/or B-cell differentiation.
Tissue specificity	Expression in mature B-cells and Burkitt lymphoma cells.
Sequence similarities	Belongs to the G-protein coupled receptor 1 family.
Cellular localization	Cell membrane.

Images



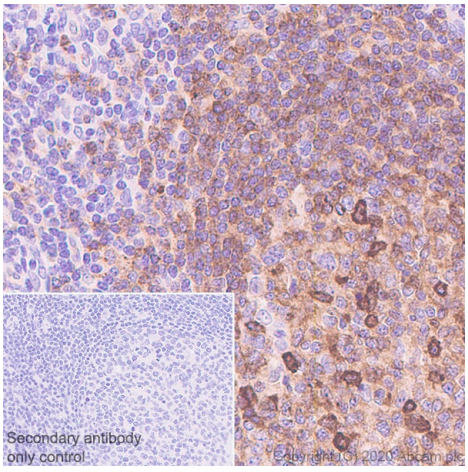
Flow Cytometry - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

Flow cytometry overlay histogram showing wild-type Raji (green line) and CXCR5 knockout Raji cells (**ab273380**) stained with ab254415 (red line). The cells were incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serumto block FC receptors and non-specific protein-protein interaction followed by the antibody (ab254415) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-rabbit IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150081**) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was Rabbit IgG (monoclonal) (**ab172730**) used at the same concentration and conditions as the primary antibody (wild-type Raji - black line; CXCR5 knockout Raji - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

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

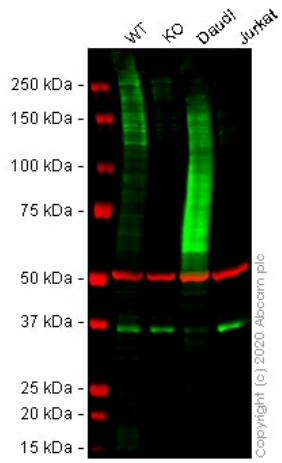


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CXCR5 with ab254415 at 1/5000 dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human tonsil (PMID: 12393412). The section was incubated with ab254415 for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Western blot - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

All lanes : Anti-CXCR5 antibody [EPR23463-30] (ab254415) at 1/1000 dilution

Lane 1 : Wild-type Raji cell lysate
Lane 2 : CXCR5 knockout Raji cell lysate
Lane 3 : Daudi cell lysate
Lane 4 : Jurkat cell lysate

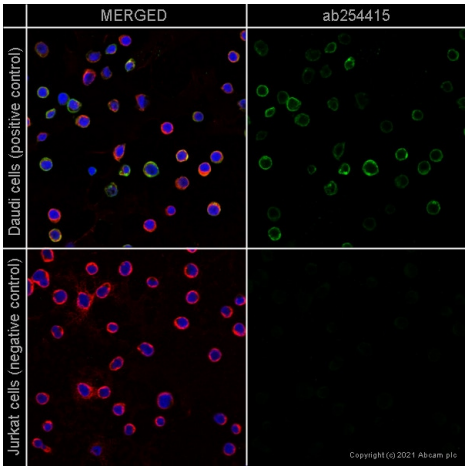
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa

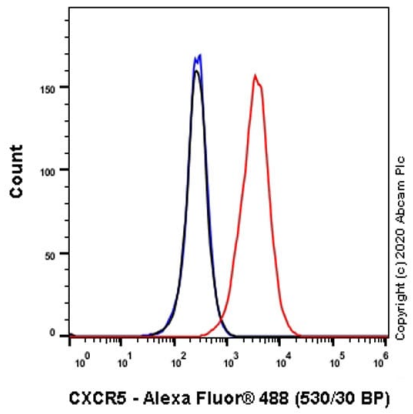
Lanes 1 - 4: Merged signal (red and green). Green - ab254415 observed at 60 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab254415 was shown to react with CXCR5 in Raji wild-type cells in Western blot with loss of signal observed in CXCR5 knockout sample. Wild-type and CXCR5 knockout Raji cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab254415 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



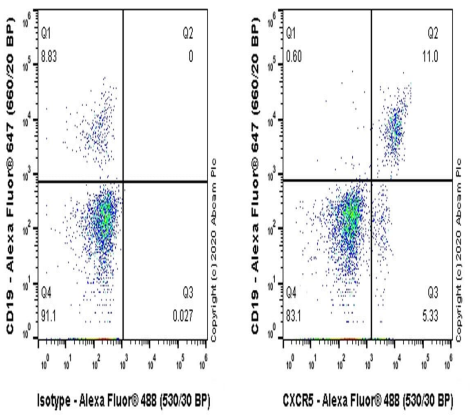
Immunocytochemistry/ Immunofluorescence - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

ab254415 staining CXCR5 in Daudi cells (top panel, positive control) and Jurkat cells (bottom panel, negative control). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes 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 with ab254415 at 5µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Flow Cytometry - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

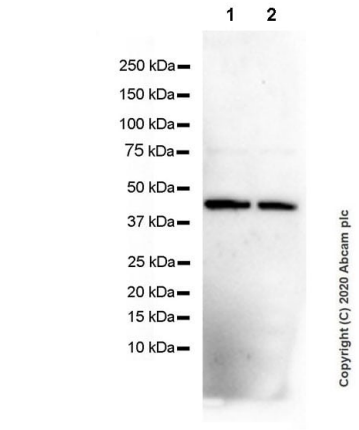
Flow cytometric analysis of Raji (Human Burkitt's lymphoma B lymphocyte) cells labelling CXCR5 with ab254415 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells.



Flow Cytometry - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

Flow cytometric analysis of human peripheral blood mononuclear cell (PBMC) cells labelling CXCR5 with ab254415 at 1/500 dilution (0.1µg) (Right) compared with a Rabbit monoclonal IgG (**ab172730**) isotype control (Left). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary at a 1/2000 dilution. Cells were stained with rabbit IgG (Left) or ab254415 (Right), then stained with anti-CD19 conjugated to Alexa Fluor® 647.

Gated on viable cells.



Western blot - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

All lanes : Anti-CXCR5 antibody [EPR23463-30] (ab254415) at 1/1000 dilution

Lane 1 : Neuro-2a (mouse neuroblastoma neuroblast), whole cell lysate
Lane 2 : A20 (mouse reticulum sarcoma B lymphocyte), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

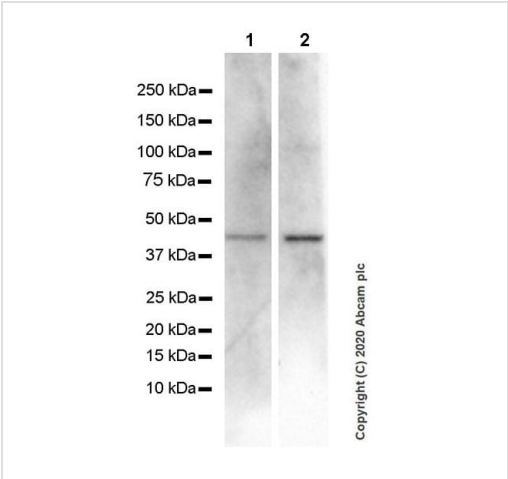
Predicted band size: 42 kDa
Observed band size: 42 kDa

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

Samples are non-boiled as boiling may cause protein aggregates.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 30553016).

Exposure time: 20 seconds.



Western blot - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

All lanes : Anti-CXCR5 antibody [EPR23463-30] (ab254415) at 1/1000 dilution

Lane 1 : Rat lymph node tissue lysate
Lane 2 : C6 (rat glial tumor glial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 42 kDa
Observed band size: 42 kDa

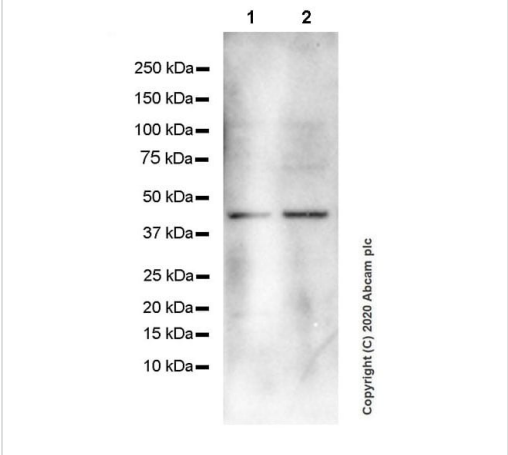
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

This blot was developed using a higher sensitivity ECL substrate.

Samples are non-boiled as boiling may cause protein aggregates.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 30553016).

Exposure time: 122 seconds.



Western blot - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

All lanes : Anti-CXCR5 antibody [EPR23463-30] (ab254415) at 1/1000 dilution

Lane 1 : Mouse spleen tissue lysate
Lane 2 : Mouse lymph node tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 42 kDa
Observed band size: 42 kDa

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

This blot was developed using a higher sensitivity ECL substrate.

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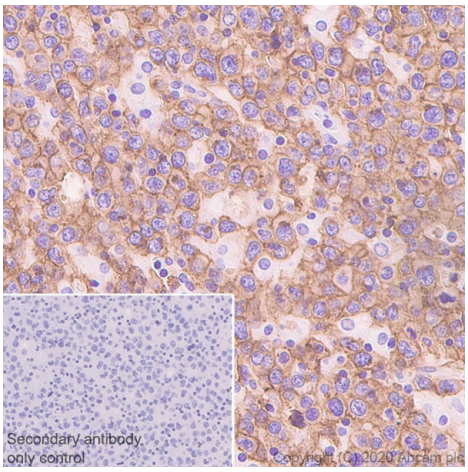

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

Immunohistochemical analysis of paraffin-embedded human diffuse large B-cell lymphoma tissue labeling CXCR5 with ab254415 at 1/5000 dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human diffuse large B-cell lymphoma. The section was incubated with ab254415 for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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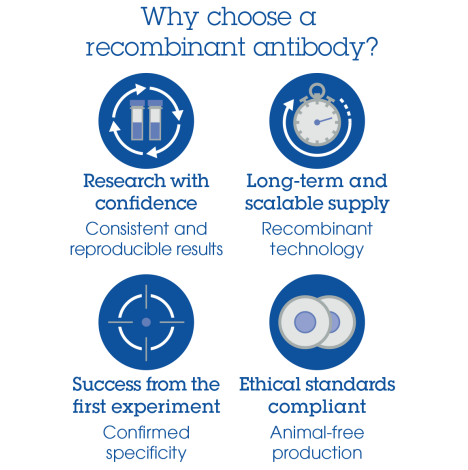

Anti-CXCR5 antibody [EPR23463-30] (ab254415)

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