abcam

ab254415

Anti-CXCR5/CD185 antibody [EPR23463-30]

Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415) is a rabbit monoclonal antibody detecting CXCR5/CD185 in **Western Blot, Flow Cytometry, IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
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	EPR23463-30
Purification technique	Affinity purification Protein A
Specificity	We observe only weak staining in human WB. We do not suggest this product for use in IHC with mouse or rat.
Concentration	0.528 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/5000
Notes	We do not suggest this product for use in IHC with mouse or rat. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Not recommended

Species	Mouse
Dilution info	-
Notes	We do not suggest this product for use in IHC with mouse or rat. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Rat
Dilution info	-
Notes	We do not suggest this product for use in IHC with mouse or rat. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

ICC/IF

Tested

Species	Human
Dilution info	5 µg/mL
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Mouse

Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1/11500
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Mouse
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000

Notes	We observe only weak staining in human WB.

Species	Rat
Dilution info	1/1000
Notes	We observe only weak staining in human WB.

Species	Human
Dilution info	1/1000
Notes	We observe only weak staining in human WB.

Target data

See full target information CXCR5/CD185

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.

Storage

Shipped at conditions	Conditional Ambient
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

What is this antibody validated in?
Anti-CXCR5 antibody [EPR23463-30] (ab254415) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Mouse, Rat samples.

What is the molecular weight of CXCR5?
Anti-CXCR5 [EPR23463-30] (ab254415) specifically detects a band for CXCR5 (UniProt: P32302) at a molecular weight of 42kDa.

Specificity confirmed
The specificity of Anti-CXCR5 antibody [EPR23463-30] (ab254415) has been confirmed by Western blot testing in CXCR5 Knockout Raji cell line, ab273380.

Other related products
We have a range of other formats of antibody clone [EPR23463-30] also available for your convenience: ab254415, Carrier free - ab272936, Oligonucleotide - ab284446, Carrier free - ab305143, PE - ab307637, APC - ab316179, FITC - ab322305

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CXCR5 also known as CD185 is a chemokine receptor with a mass of approximately 43 kDa. It is part of the G protein-coupled receptor family and is widely expressed on follicular B helper T (Tfh) cells B cells and a subset of circulating blood cells. CXCR5 is recognized as a marker for Tfh cells which are critical for the function and maturation of B cells within germinal centers. In tissues CXCR5 expression appears prominently in regions where immune cell interactions facilitate immune and inflammatory responses.
Biological function summary	CXCR5 serves a significant role in directing B cells and Tfh cells to lymphoid tissues by interacting with its ligand CXCL13. This interaction involves recruitment and organization of B cells into follicles within secondary lymphoid organs. The presence of CXCR5 allows B cells and Tfh cells to navigate the follicular zones efficiently enhance antigen-specific immune responses and contribute to long-lived humoral immunity. CXCR5 functions as part of a complex that includes signaling pathways influencing cellular movements and location.

Pathways	CXCR5 plays a significant part in the immune system's trafficking pathways particularly within the crosstalk between chemokines and their receptors. CXCR5 expression facilitates the CXC chemokine pathway which important for positioning immune cells within lymphoid organs. Additionally CXCR5 interacts with proteins such as CXCL13 working together to pattern the architecture of germinal centers where affinity maturation and class switching of antibodies occur critical processes in adaptive immunity.
Associated diseases and disorders	CXCR5 has been implicated in autoimmune diseases and certain lymphomas. For example aberrant CXCR5 expression is linked to the pathogenesis of diseases like systemic lupus erythematosus (SLE) where it might contribute to immune dysregulation and chronic inflammation. In the context of certain lymphomas CXCR5's role in lymphoid organ architecture and cell migration becomes even more relevant. Its expression often parallels the presence of CXCL13 hinting at a network of interactions promoting tumor survival and growth within lymphoid landscapes. Understanding these relationships can offer insight into developing therapeutic strategies potentially involving CXCR5 inhibitors or anti-CXCR5 antibodies to address these diseases.

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

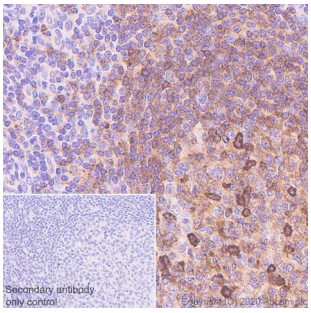
We do not recommend this combination. It is not covered by our product promise.

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.

11 product images

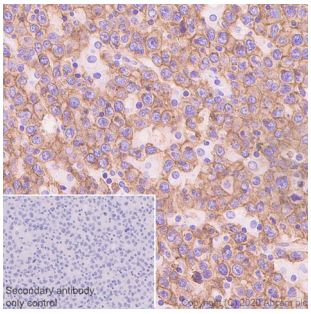


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CXCR5/CD185 antibody

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.

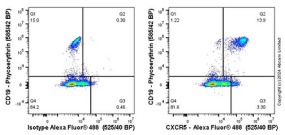


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CXCR5/CD185 antibody

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).

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



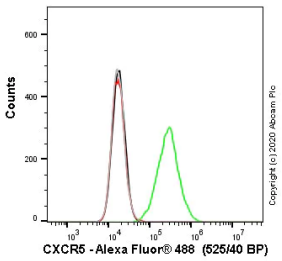
Flow Cytometry - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Flow Cytometry staining of human peripheral blood mononuclear cells (PBMCs) using rabbit Anti-CXCR5/CD185 antibody

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab254415 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (left). PBMCs were incubated for 30 mins on ice in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab254415 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (1x 10^6 in 100 µl at 0.2 µg/ml (1/11500 dilution)) for 30 mins on ice. The cells were simultaneously stained with CD19.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 dilution for 30 mins on ice

Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on live cells.



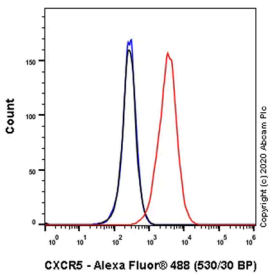
Flow Cytometry - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Flow Cytometry staining using rabbit Anti-CXCR5/CD185 antibody

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).

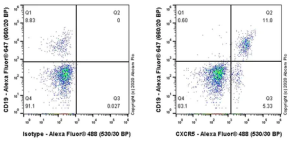
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Flow Cytometry - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Flow Cytometry staining using rabbit Anti-CXCR5/CD185 antibody

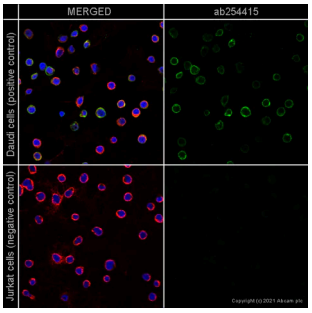
Flow cytometric analysis of Raji (Human Burkitt's lymphoma B lymphocyte) cells labelling CXCR5 with ab254415 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary 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/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Flow Cytometry staining using rabbit Anti-CXCR5/CD185 antibody

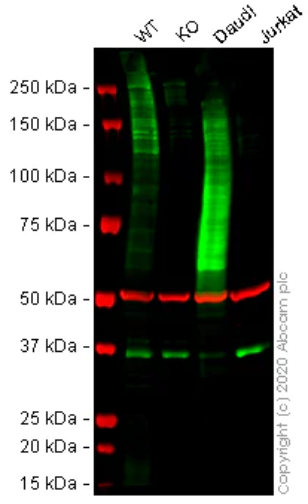
Flow cytometric analysis of human peripheral blood mononuclear cell (PBMC) cells labelling CXCR5 with ab254415 at 1/500 dilution (0.1µg) (Right) compared with a Rabbit monoclonal IgG (ab172730) isotype control (Left). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary at a 1/2000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



Immunocytochemistry/ Immunofluorescence - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CXCR5/CD185 antibody

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).



Western blot - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Western blot staining using rabbit Anti-CXCR5/CD185 antibody

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.

All lanes:
Western blot - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415) at 1/1000 dilution

Lane 1:
Wild-type Raji cell lysate at 30 µg

Lane 2:
CXCR5 knockout Raji cell lysate at 30 µg

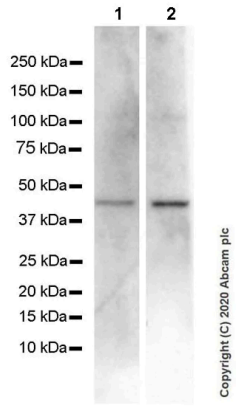
Lane 2:
Western blot - Human CXCR5 knockout Raji cell line (ab273380)

Lane 3:
Daudi cell lysate at 30 µg

Lane 4:
Jurkat cell lysate at 30 µg

Performed under reducing conditions.

Predicted band size: 42 kDa



Western blot - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Western blot staining using rabbit Anti-CXCR5/CD185 antibody

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.

All lanes:
Western blot - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415) at 1/1000 dilution

Lane 1:
Rat lymph node tissue lysate at 20 µg

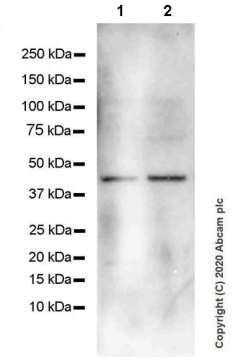
Lane 2:
C6 (rat glial tumor glial cell), whole cell lysate at 20 µg

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



Western blot - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Western blot staining using rabbit Anti-CXCR5/CD185 antibody

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.

All lanes:
Western blot - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415) at 1/1000 dilution

Lane 1:
Mouse spleen tissue lysate at 20 µg

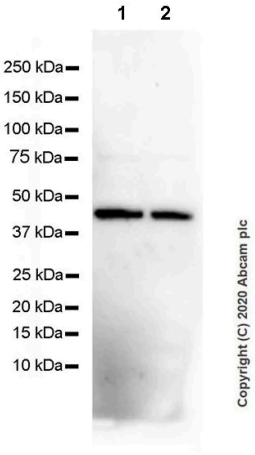
Lane 2:
Mouse lymph node tissue lysate at 20 µg

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



Western blot - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415)

CXCR5/CD185 Western blot staining using rabbit Anti-CXCR5/CD185 antibody

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.

All lanes:
Western blot - Anti-CXCR5/CD185 antibody [EPR23463-30] (ab254415) at 1/1000 dilution

Lane 1:
Neuro-2a (mouse neuroblastoma neuroblast), whole cell lysate at 20 µg

Lane 2:
A20 (mouse reticulum sarcoma B lymphocyte), whole cell lysate at 20 µg

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

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