

### Anti-CXCR5 antibody [EPR23463-30] ab254415

敲除验证
重组
RabMAb

★★★★☆
[1 Abreviews](#)
[1 References](#)
[11 图像](#)

#### 概述

产品名称	Anti-CXCR5抗体[EPR23463-30]
描述	兔单克隆抗体[EPR23463-30] to CXCR5
宿主	Rabbit
特异性	We observe only weak staining in human WB. We do not suggest this product for use in IHC with mouse or rat.
经测试应用	<b>适用于:</b> ICC/IF, WB, Flow Cyt, IHC-P <b>不适用于:</b> IP
种属反应性	<b>与反应:</b> Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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.
常规说明	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### 性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR23463-30
同种型	IgG

## 应用

## The Abpromise guarantee Abpromise™承诺保证使用ab254415于以下的经测试应用

“应用说明”部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

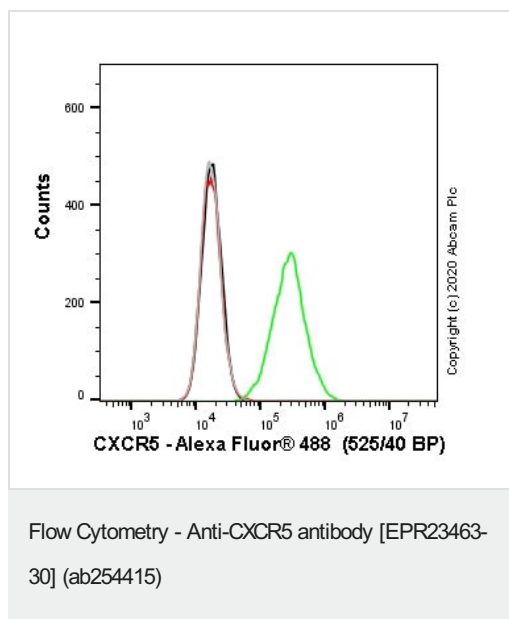
应用	Ab评论	说明
ICC/IF		Use a concentration of 5 µg/ml.
WB		1/1000. Predicted molecular weight: 42 kDa. We observe only weak staining in human WB.
Flow Cyt	★★★★☆ (1)	1/500.
IHC-P		1/5000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. We do not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

应用说明 Is unsuitable for IP.

## 靶标

功能	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.
组织特异性	Expression in mature B-cells and Burkitt lymphoma cells.
序列相似性	Belongs to the G-protein coupled receptor 1 family.
细胞定位	Cell membrane.

图片

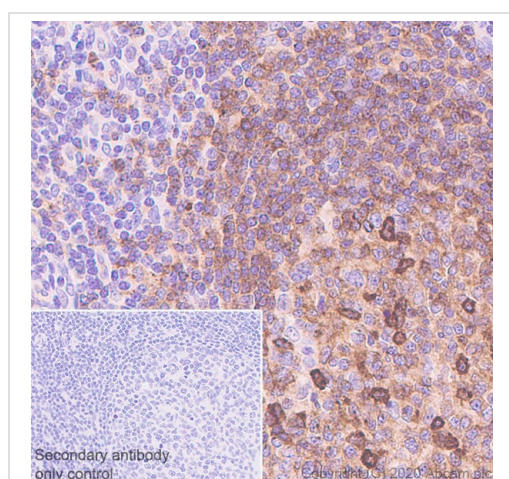


Flow cytometry overlay histogram showing wild-type Raji (green line) and CXCR5 knockout Raji cells (**ab273380**) stained with ab254415 (red line). The cells were incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab254415) ( $1 \times 10^6$  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.

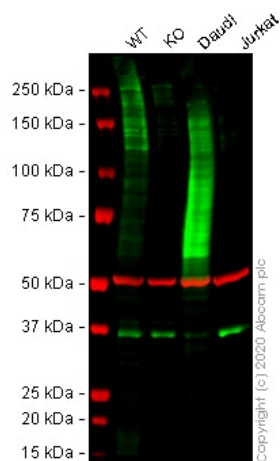


Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CXCR5 with ab254415 at 1/5000 dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human tonsil (PMID: 12393412).

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

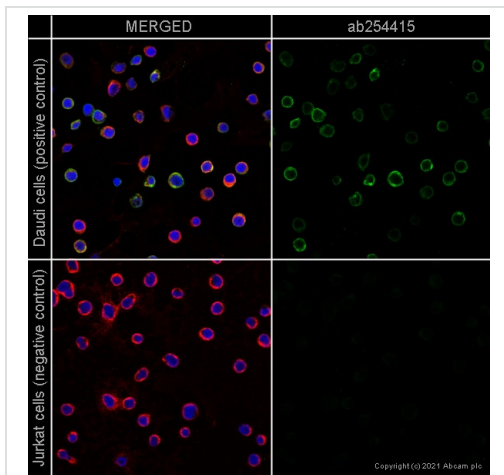
Lysates/proteins at 30 µg per lane.

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.

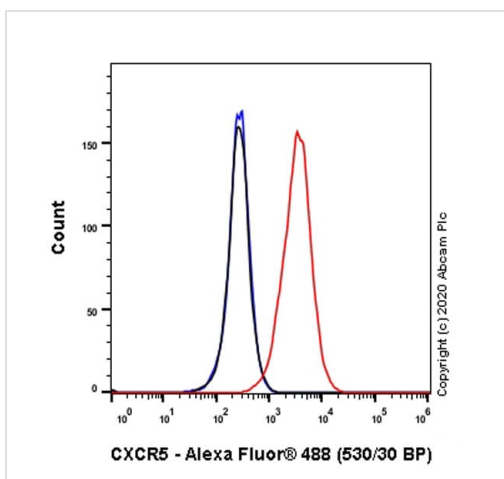
ab254415 was shown to react with CXCR5 in Raji wild-type cells in Western blot with loss of signal observed in CXCR5 knockout sample. Wild-type and CXCR5 knockout Raji cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation 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)

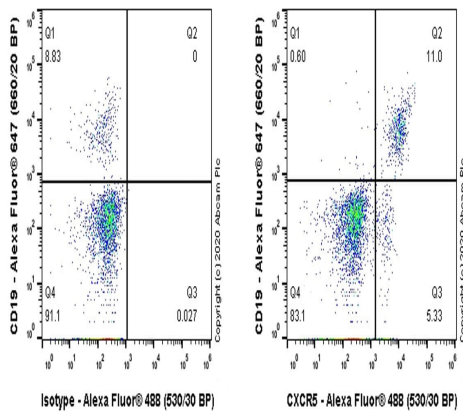
ab254415 staining CXCR5 in Daudi cells (top panel, positive control) and Jurkat cells (bottom panel, negative control). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% 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).

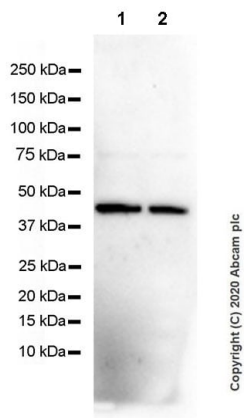


Flow Cytometry - Anti-CXCR5 antibody [EPR23463-30] (ab254415)

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)



Western blot - 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 (0.1 µg) (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.

**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

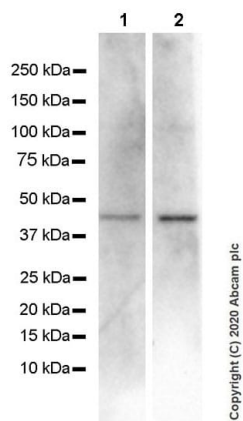
**Observed band size:** 42 kDa

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

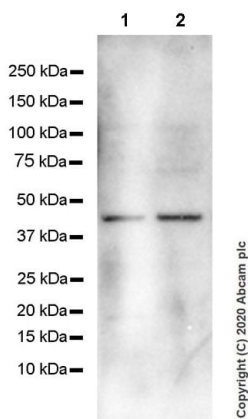
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

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

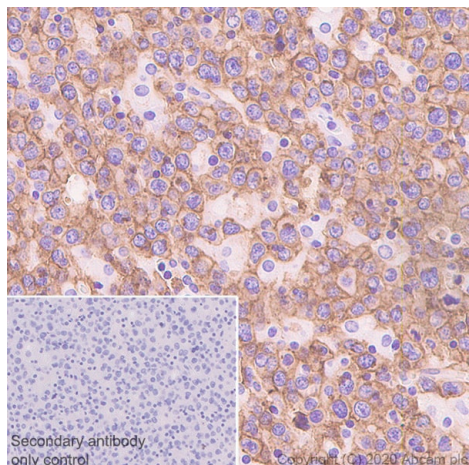
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

This blot was developed using a higher sensitivity ECL substrate.

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.



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**).

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

#### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

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

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

**Our Abpromise to you: Quality guaranteed and expert technical support**

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.cn/abpromise> or contact our technical team.

#### **Terms and conditions**

---

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors