abcam

Product datasheet

Anti-CXCR4 antibody [EPUMBR3] ab181020

KO 評価済 リコンビナント RabMAb

35 References 画像数 9

製品の概要

製品名	Anti-CXCR4 antibody [EPUMBR3]
製品の詳細	Rabbit monoclonal [EPUMBR3] to CXCR4
由来種	Rabbit
特異性	This antibody recognizes only the non-phosphorylated C-terminus of CXCR4 (residues 341-352). Phosphorylation of S346/347 blocks antibody binding. PMID: 24154522, 25451233. We recommend dephosphorylation of samples using lambda phosphatase treatment. Please refer to application notes.
アプリケーション	**適用あり:** Flow Cyt (Intra), WB, IHC-P, ICC/IF **適用なし:** IP
種交差性	**交差種:** Mouse, Human, Recombinant fragment
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab256223**)
ポジティブ・コントロール	WB: Jurkat whole cell lysate. IF/ICC: Jurkat and Ramos cells. IHC-P: Retina and brain of E14 mouse embryo, Human small cell lung carcinoma tissue. Flow Cyt (intra): Jurkat cells.
特記事項	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**.

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: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPUMBR3
アイソタイプ	IgG

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ab181020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
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000 - 1/10000. Predicted molecular weight: 39 kDa.Can be blocked with **CXCR4 peptide (ab256223)**. We recommend lambda protein phosphatase treatment of the membrane prior to primary antibody incubation (PMID 24154522). Use 800U for 1 hr at RT then rinse in wash buffer three times.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol. Boil slides seven times for five minutes each in pH 6 citrate buffer. We recommend lambda protein phosphatase treatment prior to IHC processing (PMID 24154522). Use 800U for 1 hr at RT then rinse in PBS three times.
ICC/IF		1/500. **For unpurified use at 5 µg/mL.**

追加情報 Is unsuitable for IP.

ターゲット情報

機能 Receptor for the C-X-C chemokine CXCL12/SDF-1 that transduces a signal by increasing intracellular calcium ions levels and enhancing MAPK1/MAPK3 activation. Acts as a receptor for extracellular ubiquitin; leading to enhance intracellular calcium ions and reduce cellular cAMP levels. Involved in haematopoiesis and in cardiac ventricular septum formation. Plays also an essential role in vascularization of the gastrointestinal tract, probably by regulating vascular branching and/or remodeling processes in endothelial cells. Could be involved in cerebellar development. In the CNS, could mediate hippocampal-neuron survival. Acts as a coreceptor (CD4 being the primary receptor) for HIV-1 X4 isolates and as a primary receptor for some HIV-2 isolates. Promotes Env-mediated fusion of the virus.

組織特異性 Expressed in numerous tissues, such as peripheral blood leukocytes, spleen, thymus, spinal cord, heart, placenta, lung, liver, skeletal muscle, kidney, pancreas, cerebellum, cerebral cortex and medulla (in microglia as well as in astrocytes), brain microvascular, coronary artery and umbilical cord endothelial cells. Isoform 1 is predominant in all tissues tested.

関連疾患

Defects in CXCR4 are a cause of WHIM syndrome (WHIM) [MIM:193670]; also known as warts, hypogammaglobulinemia, infections and myelokathexis. WHIM syndrome is an immunodeficiency disease characterized by neutropenia, hypogammaglobulinemia and extensive human papillomavirus (HPV) infection. Despite the peripheral neutropenia, bone marrow aspirates from affected individuals contain abundant mature myeloid cells, a condition termed myelokathexis.

配列類似性

Belongs to the G-protein coupled receptor 1 family.

ドメイン

The amino-terminus is critical for ligand binding. Residues in all four extracellular regions contribute to HIV-1 coreceptor activity.

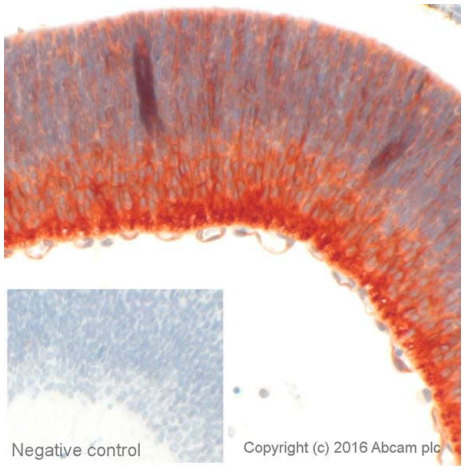
翻訳後修飾

Phosphorylated on agonist stimulation. Rapidly phosphorylated on serine and threonine residues in the C-terminal. Phosphorylation at Ser-324 and Ser-325 leads to recruitment of ITCH, ubiquitination and protein degradation.
Ubiquitinated by ITCH at the cell membrane on agonist stimulation. The ubiquitin-dependent mechanism, endosomal sorting complex required for transport (ESCRT), then targets CXCR4 for lysosomal degradation. This process is dependent also on prior Ser-/Thr-phosphorylation in the C-terminal of CXCR4. Also binding of ARRB1 to STAM negatively regulates CXCR4 sorting to lysosomes though modulating ubiquitination of SFR5S.
Sulfation on Tyr-21 is required for efficient binding of CXCL12/SDF-1alpha and promotes its dimerization.
O- and N-glycosylated. Asn-11 is the principal site of N-glycosylation. There appears to be very little or no glycosylation on Asn-176. N-glycosylation masks coreceptor function in both X4 and R5 laboratory-adapted and primary HIV-1 strains through inhibiting interaction with their Env glycoproteins. The O-glycosylation chondroitin sulfate attachment does not affect interaction with CXCL12/SDF-1alpha nor its coreceptor activity.

細胞内局在

Cell membrane. In unstimulated cells, diffuse pattern on plasma membrane. On agonist stimulation, colocalizes with ITCH at the plasma membrane where it becomes ubiquitinated.

画像

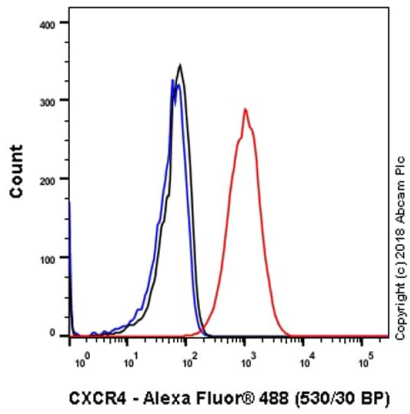


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [EPUMBR3] (ab181020)

This experiment was carried out by an anonymous collaborator

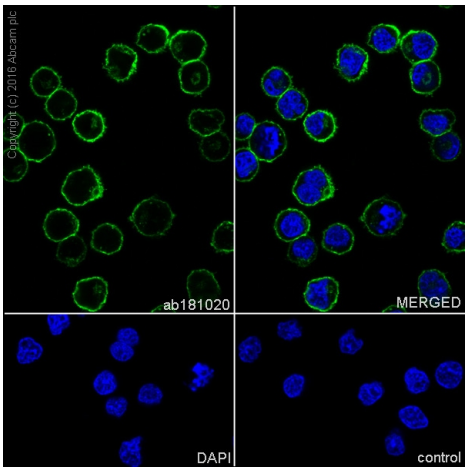
IHC image of CXCR4 staining on retina of E14 mouse embryo formalin fixed paraffin embedded tissue section. The section was pre-treated using heat mediated antigen retrieval with 10 mM sodium citrate buffer (pH6) for 20 mins in a microwave at 600W, and incubated overnight at + 4^{O}C with ab181020 at 5 ugml. Staining of primary antibody was detected using the appropriate biotinylated secondary antibodies followed by incubation with avidin-biotinylated peroxidase solution. DAB was used as the chromogen (15 min). The section was then counterstained with haematoxylin. As a negative control (inset), an identical assay was performed on retina of E14 knockout mouse (CXCR4 -/-) embryo.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



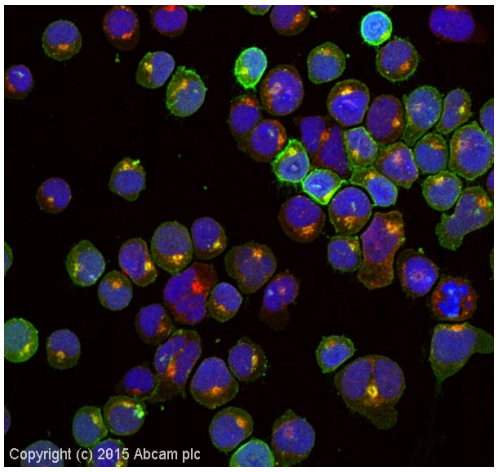
Flow Cytometry (Intracellular) - Anti-CXCR4 antibody [EPUMBR3] (ab181020)

Intracellular Flow Cytometry analysis of Jurkat (human T cell leukemia T lymphocyte) cells labeling CXCR4 with purified ab181020 at 1/200 dilution (10.23 µg/ml) - Red. Cells were fixed with 4% paraformaldehyde . A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) - Black. Unlabeled control - Blue. Untreated cells - Green



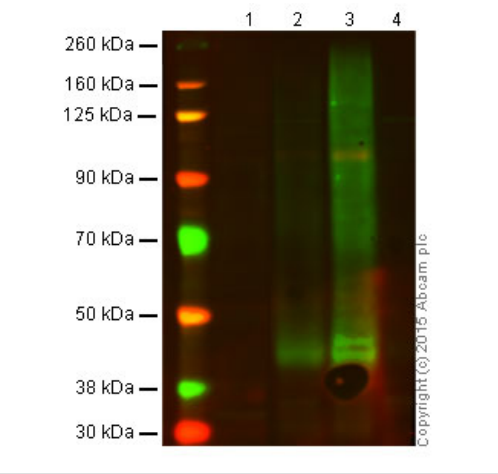
Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [EPUMBR3] (ab181020)

Immunocytochemistry/Immunofluorescence analysis of Ramos (Human Burkitt's lymphoma cell line) labeling CXCR4 with purified ab181020 at 1/500 dilution. Cells were fixed with 4% PFA and permeabilized with 0.1% tritonX-100. **ab150077** Goat anti rabbit IgG (Alexa Fluor® 488) at 1/1000 was used as the secondary antibody. Nuclei were counterstained with DAPI. PBS was used instead of the primary antibody as the negative control.



Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [EPUMBR3] (ab181020)

ab181020 stained Jurkat cells. The cells were 100% methanol fixed for 5 minutes at -20°C and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab181020 at 5µg/ml) overnight at +4°C. The secondary antibody (pseudo-colored green) was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150081**) used at a 1/1000 dilution for 1hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Western blot - Anti-CXCR4 antibody [EPUMBR3] (ab181020)

All lanes : Anti-CXCR4 antibody [EPUMBR3] (ab181020)

Lane 1 : CHO (chinese hamster ovary cell line) whole cell lysate (negative control)
Lane 2 : Jurkat whole cell
Lane 3 : Jurkat membrane
Lane 4 : Jurkat nuclear (negative control)

Lysates/proteins at 20 µg per lane.

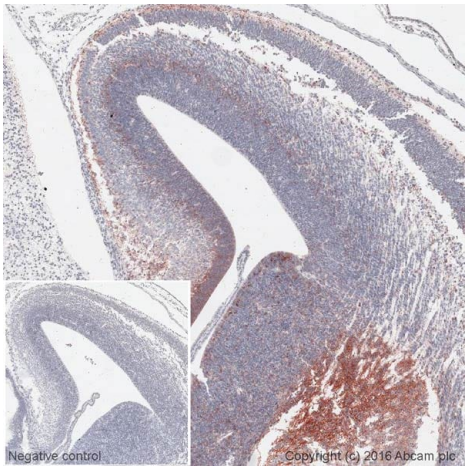
Secondary
All lanes : Goat anti-rabbit at 1/10000 dilution

Predicted band size: 39 kDa
Observed band size: 43 kDa

Running buffer: MOPS.

Conditions: denatured/reduced.

This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with ab181020 (anti-CXCR4) and **ab7671** (loading ctrl), overnight at 4°C. Before imaging, antibody binding was detected using labelled goat anti-rabbit (H+L; green) and labelled goat anti-mouse (H+L; red) at 1:10,000 dilutions for 1hr at room temperature.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [EPUMBR3] (ab181020)

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IHC image of CXCR4 staining on Brain of E14 mouse embryo formalin fixed paraffin embedded tissue section. The section was pre-treated using heat mediated antigen retrieval with 10 mM sodium citrate buffer (pH6) for 20 mins in a microwave at 600W, and incubated overnight at + 4^{o}C with ab181020 at 1/500 dilution. Staining of primary antibody was detected using the appropriate biotinylated secondary antibodies followed by incubation with avidin-biotinylated peroxidase solution. DAB was used as the chromogen (15 min). The section was then counterstained with haematoxylin. As a negative control (inset), an identical assay was performed on Brain of E14 knockout mouse (CXCR4 -/-) embryo.

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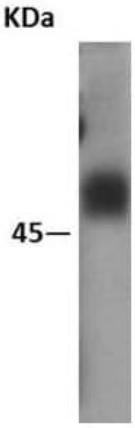
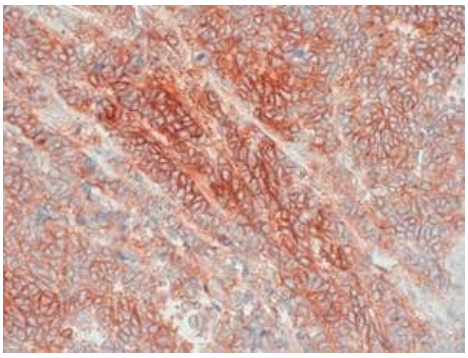


Western blot - Anti-CXCR4 antibody [EPUMBR3] (ab181020)

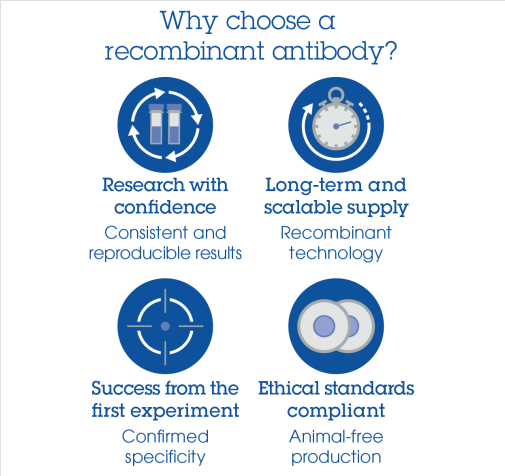
Anti-CXCR4 antibody [EPUMBR3] (ab181020) at 1/1000 dilution + CXCR4 stably expressed in HEK293 cells

Predicted band size: 39 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [EPUMBR3] (ab181020)

Immunohistochemical analysis of paraffin embedded Human small cell lung carcinoma tissue labeling CXCR4 using ab181020.



Anti-CXCR4 antibody [EPUMBR3] (ab181020)

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