abcam

Product datasheet

Anti-CXCR4 antibody [EPUMBR3] - Low endotoxin, Azide free ab222223

KO VALIDATED Recombinant RabMAb

7 Images

Overview

Product name	Anti-CXCR4 antibody [EPUMBR3] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPUMBR3] to CXCR4 - Low endotoxin, Azide free
Host species	Rabbit
Specificity	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.
Tested applications	**Suitable for:** WB, Flow Cyt (Intra), IHC-P, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Human, Recombinant fragment
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Human CXCR4 stably expressed in HEK293 cells. Human small cell lung carcinoma tissue.IF/ICC: Jurkat and Ramos cells. IHC-P: FFPE retina of mouse E14 embryo. Flow Cyt (intra): Jurkat cells.
General notes	ab222223 is the carrier-free version of **ab181020**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPUMBR3
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab222223 in the following tested applications.

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

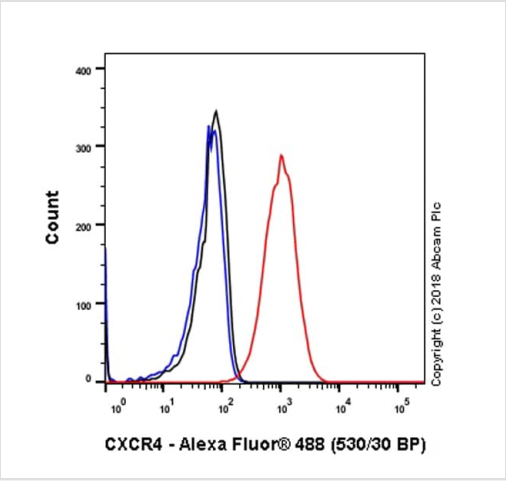
Application	Abreviews	Notes
WB		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol. Boil slides 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		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

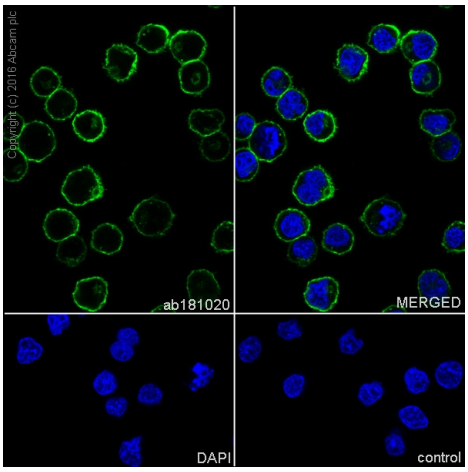
Function	Receptor for the C-X-C chemokine CXCL12/SDF-1 that transduces a signal by increasing intracellular calcium ions levels and enhancing MAPK1/MAPK3 activation. Acts as a receptor for extracellular ubiquitin; leading to enhance intracellular calcium ions and reduce cellular cAMP levels. Involved 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.
Tissue specificity	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.
Involvement in disease	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.
Sequence similarities	Belongs to the G-protein coupled receptor 1 family.
Domain	The amino-terminus is critical for ligand binding. Residues in all four extracellular regions contribute to HIV-1 coreceptor activity.
Post-translational modifications	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.
Cellular localization	Cell membrane. In unstimulated cells, diffuse pattern on plasma membrane. On agonist stimulation, colocalizes with ITCH at the plasma membrane where it becomes ubiquitinated.

Images



Flow Cytometry (Intracellular) - Anti-CXCR4 antibody [EPUMBR3] - Low endotoxin, Azide free (ab222223)

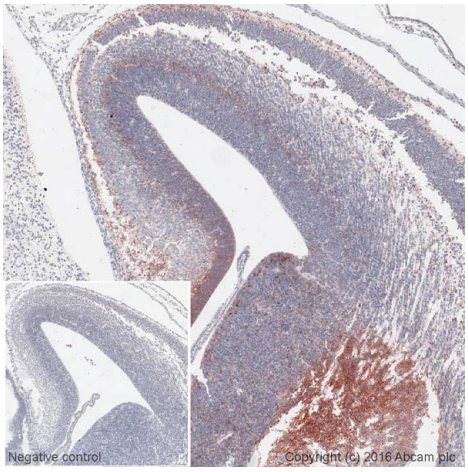
Intracellular Flow Cytometry analysis of Jurkat (human T cell leukemia T lymphocyte) cells labeling CXCR4 with purified **ab181020** at 1/200 dilution (10.23 µg/ml) - Red. Cells were fixed with 4% paraformaldehyde . A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) - Black. Unlabeled control - Blue. Untreated cells - GreenThis data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab222223)



Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [EPUMBR3] - Low endotoxin, Azide free (ab222223)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181020**).



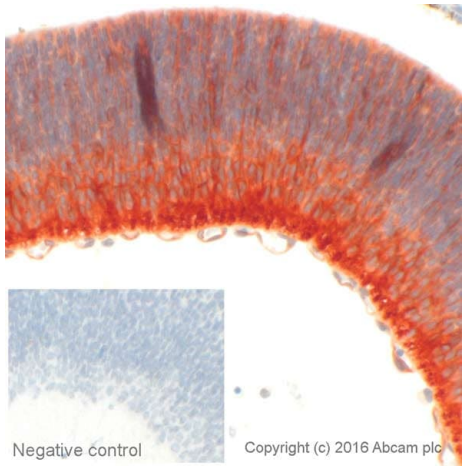
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [EPUMBR3] - Low endotoxin, Azide free (ab222223)

This experiment was carried out by an anonymous collaborator

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.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181020**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [EPUMBR3] - Low endotoxin, Azide free (ab222223)

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.

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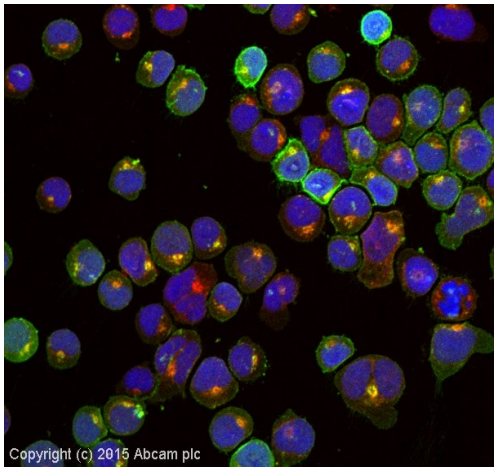
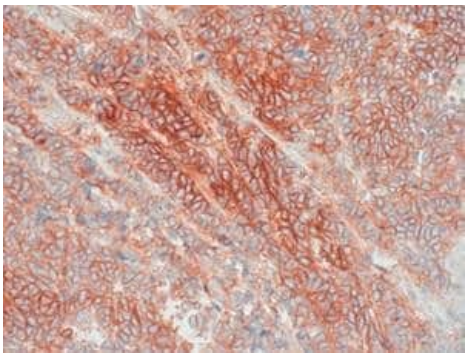


Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [EPUMBR3] - Low endotoxin, Azide free (ab222223)

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.

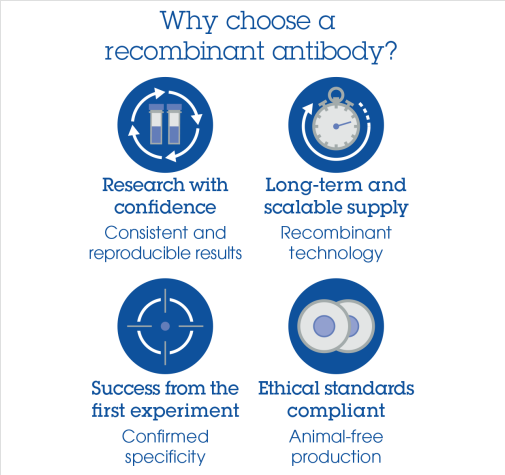
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181020**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [EPUMBR3] - Low endotoxin, Azide free (ab222223)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181020**).



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