abcam

Product datasheet

Anti-CXCR4 antibody [UMB2] - BSA and Azide free ab197203

Recombinant RabMAb

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Overview

Product name	Anti-CXCR4 antibody [UMB2] - BSA and Azide free
Description	Rabbit monoclonal [UMB2] to CXCR4 - BSA and Azide free
Host species	Rabbit
Specificity	Although some customers can get this ab to work in mouse and rat successfully we cannot reproduce this in house in IHC so cannot guarantee it. We would recommend antibody Anti-CXCR4 antibody [EPUMBR3] (**ab181020**) for use in mouse IHC. 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:** IHC-Fr, IHC-P, Flow Cyt (Intra), ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab155072**)
Positive control	WB: HeLa, Jurkat and WI-38 cell lysates; HEK239 transfected with CXCR4, cell lysate. ICC/IF: Jurkat cells. IHC-P: Human cervical carcinoma, bladder cancer tissue, ovarian adenocarcinoma tissue and tonsil tissue. Flow Cyt (intra): Jurkat cells, HEK-293T cells transfected with human CXCR4 expressed vector. IHC-Fr: Mouse and rat E14.5 cerebrum
General notes	ab197203 is the carrier-free version of **ab124824**. 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.

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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	UMB2
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab197203 in the following tested applications.

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

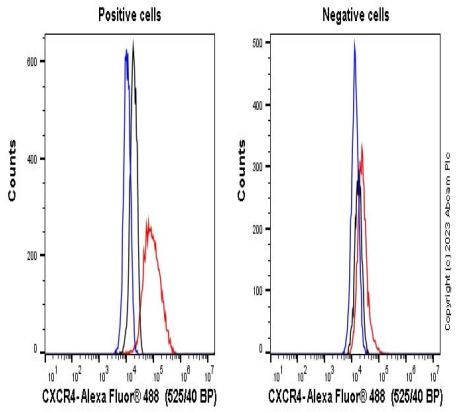
Application	Abreviews	Notes
IHC-Fr		Use at an assay dependent concentration.
IHC-P		1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 39 kDa. Please check the parent abID, **ab124824**, for more information on dilution ranges.

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 [UMB2] - BSA and Azide free (ab197203)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124824**).

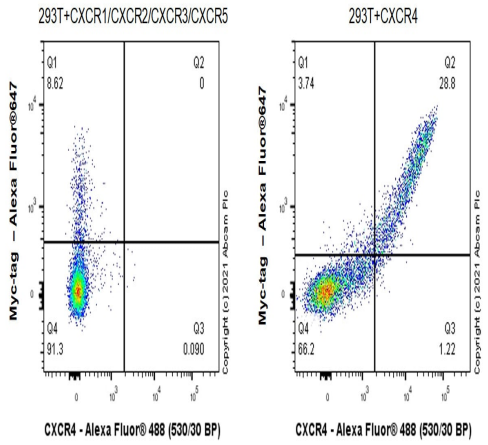
Flow cytometry overlay histogram showing left Jurkat positive cells and right negative HepG2 stained with **ab124824** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab124824**) (1x 10^6 in 100µl at 1.0µg/ml (1/1900)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

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

This antibody gave a positive signal in Jurkat Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

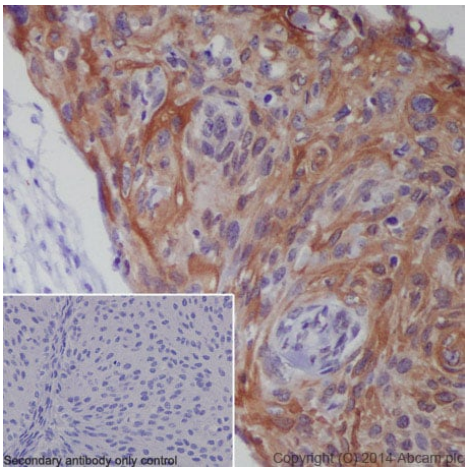


Flow Cytometry (Intracellular) - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124824**).

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HEK-293T (human embryonic kidney) cells transfected with a mix of human CXCR1, CXCR2, CXCR3 and CXCR5 expression vector containing a myc-his tag (Left) / HEK-293T transfected with a human CXCR4 expression vector containing a myc-his tag (Right) labelling CXCR4 with **ab124824** at 1/2000 dilution (0.1µg)/ Left and Right. A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/3000 dilution was used as the secondary antibody.

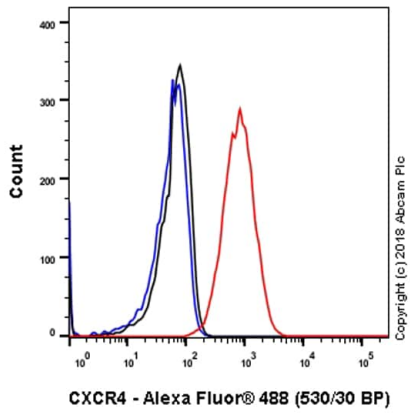
No cross-reactivity with CXCR1/CXCR2/CXCR3/CXCR5.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

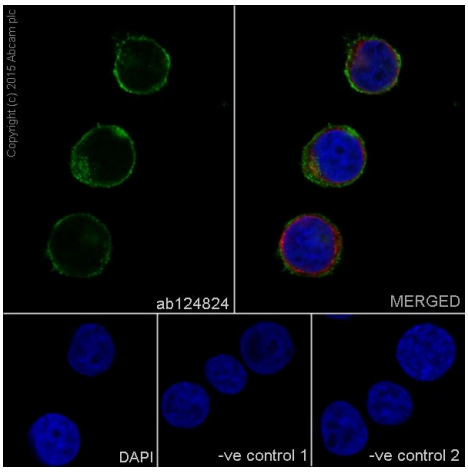
Immunohistochemical staining of paraffin embedded human bladder cancer with purified **ab124824** at a working dilution of 1/500. The secondary antibody used is **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124824**).



Flow Cytometry (Intracellular) - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

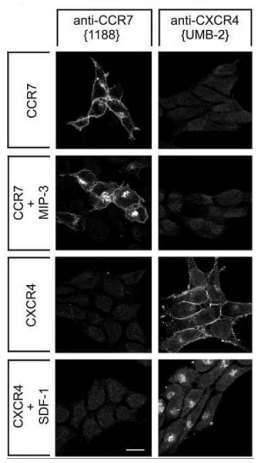
Intracellular Flow Cytometry analysis of Jurkat (human T cell leukemia T lymphocyte) cells labeling CXCR4 with purified **ab124824** at 1/260 dilution (10 µg/ml) - Red. Cells were fixed with 4% paraformaldehyde . A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000 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 (ab197203)



Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Immunofluorescence staining of Jurkat cells with purified **ab124824** at a working dilution of 1 in 250, counter-stained with DAPI. Tubulin was stained with mouse anti-tubulin at a dilution of 1/1000 (**ab7291**) and Alexa Fluor® 594 goat anti-mouse at a dilution of 1/500 (**ab150120**) . The secondary antibody was **ab150077** Alexa Fluor® 488 goat anti rabbit, used at a dilution of 1 in 500. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in the bottom middle and right hand panels - for the first negative control, purified **ab124824** was used at a dilution of 1/200 followed by an Alexa Fluor® 555 goat anti-mouse antibody at a dilution of 1/500 and for the second negative control mouse primary antibody (**ab7291**) and anti-rabbit secondary antibody (**ab15007**) were used.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124824**).

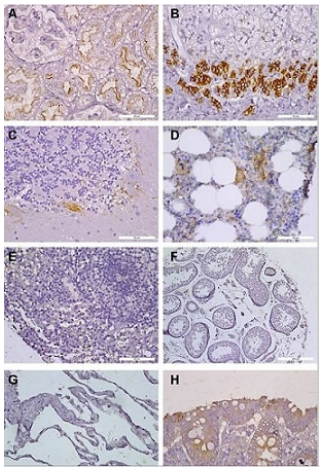


Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Fischer T. et al. PLoS One. 2008;3(12):e4069. doi: 10.1371/journal.pone.0004069. Epub 2008 Dec 31.

Characterization of UMB-2 (**ab124824**) by immunofluorescent staining of transfected cells. HEK-293 cells expressing CCR7 or CXCR4 were either not exposed or exposed to 100 ng/ml MIP-3 or 100 ng/ml SDF-1 for 30 min, subsequently fixed and immunofluorescently stained with 1 µg/ml anti-CCR7 {1188} or anti-CXCR4 {UMB-2} at a dilution of 1:100. Note that UMB-2 detected prominent immunofluorescence at the level of the plasma membrane only in CXCR4- but not in CCR7-expressing cells, and that SDF-1 exposure induced a rapid translocation of CXCR4 receptor immunostaining from the plasma membrane into the cytosol. Representative results from one of three independent experiments are shown. Scale bar, 20 µm.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124824**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Costa MJ. et al. PLoS One. 2018 Mar 19;13(3):e0194688. doi: 10.1371/journal.pone.0194688. eCollection 2018.

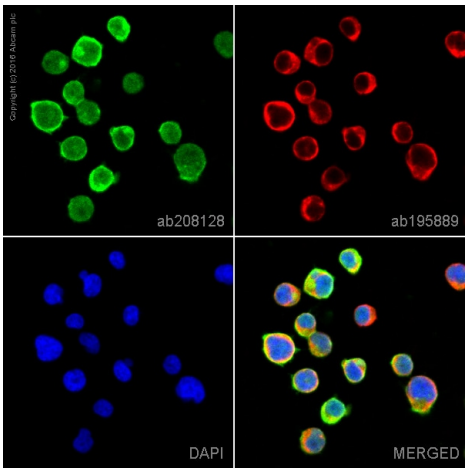
Immunohistochemical detection of CXCR4 expression in human tissue specimens of normal appearance

CXCR4 was detected in the indicated PFA-fixed, paraffin-embedded human tissues using **ab124824** at 5 µ/ml overnight at 4°C.

A, kidney. B, adrenal gland. C, cerebellum. D, bone marrow, brown staining: CXCR4, green staining: CD45. E, Spleen. F, testis. G, lung. H, colon.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124824**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



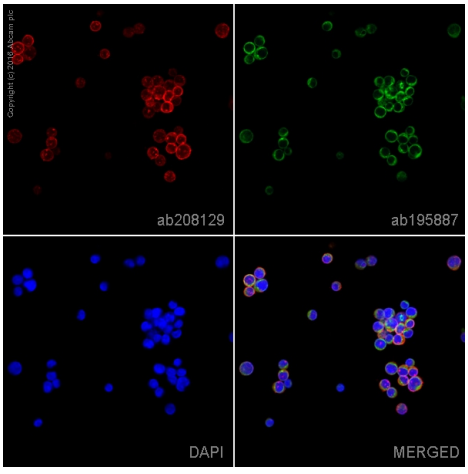
Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Clone UMB2 (ab197203) has been successfully conjugated by Abcam. This image was generated using Anti-CXCR4 antibody [UMB2] (Alexa Fluor® 488). Please refer to **ab208128** for protocol details.

ab208128 staining CXCR4 in Jurkat cells. The cells were fixed with 4% formaldehyde (10 min) and then incubated in 1%BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated overnight at +4°C with **ab208128** at 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in Jurkat cells fixed with 80% methanol (5 min).



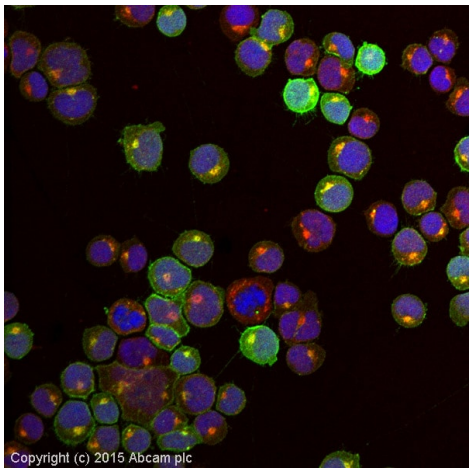
Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Clone UMB2 (ab197203) has been successfully conjugated by Abcam. This image was generated using Anti-CXCR4 antibody [UMB2] (Alexa Fluor® 647). Please refer to **ab208129** for protocol details.

ab208129 staining CXCR4 in Jurkat cells. The cells were fixed with 80% methanol (5min), 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 overnight at +4°C with **ab208129** at 1/50 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

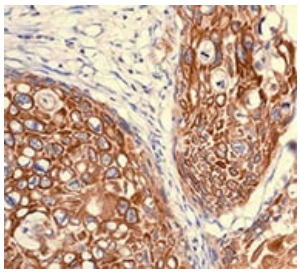
This product also gave a positive signal under the same testing conditions in Jurkat cells fixed with 4% formaldehyde (10 min).



Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

ab124824 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 (**ab124824** at 5ug/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 (**ab124824**).

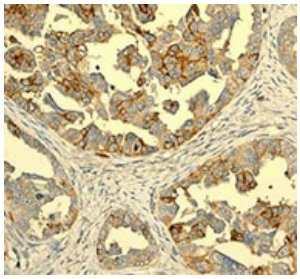


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Unpurified **ab124824**, at 1/50 dilution, staining CXCR4 in paraffin-embedded Human cervical carcinoma tissue by immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124824**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

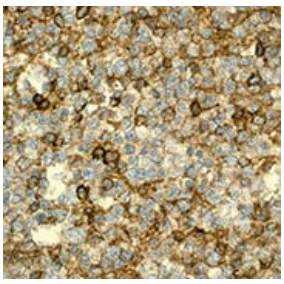


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Unpurified **ab124824**, at 1/50 dilution, staining CXCR4 in paraffin-embedded Human ovarian adenocarcinoma tissue by immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124824**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

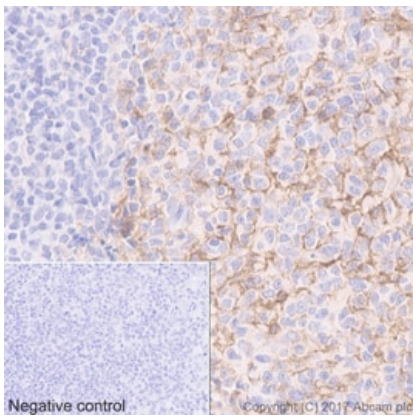


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Unpurified **ab124824**, at 1/50 dilution, staining CXCR4 in paraffin-embedded Human tonsil tissue by immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124824**).

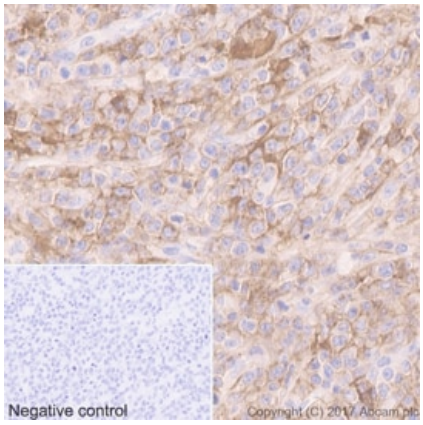
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue sections labeling CXCR4 with purified ab197203 at 1/1000 dilution (1.067 µg/ml). Heat mediated antigen retrieval was performed using Tris/EDTA Buffer, PH9 (**ab93684**). Hematoxylin was used to counter stain. Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used.

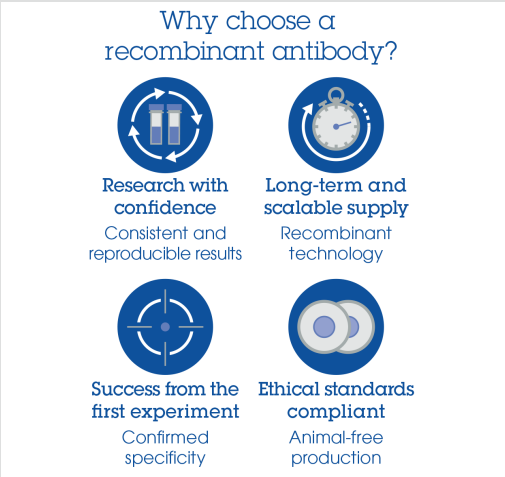
Membranous with weak cytoplasmic staining on human tonsil.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human B-cell non-hodgkin lymphoma tissue sections labeling CXCR4 with purified ab197203 at 1/1000 dilution (1.067 µg/ml). Heat mediated antigen retrieval was performed using Tris/EDTA Buffer, PH9 (**ab93684**). Hematoxylin was used to counter stain. Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used.

Cytoplasmic and membranous staining on tumor cells of human B-cell non-Hodgkin lymphoma.



Anti-CXCR4 antibody [UMB2] - BSA and Azide free (ab197203)

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