

Product datasheet

Anti-CXCR4 antibody [UMB2] ab124824

Recombinant RabMAb

★★★★★ 17 Abreviews 217 References 20 Images

Overview

Product name	Anti-CXCR4 antibody [UMB2]
Description	Rabbit monoclonal [UMB2] to CXCR4
Host species	Rabbit
Specificity	<p>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.</p> <p>This antibody recognizes only the non-phosphorylated C-terminus of CXCR4 (residues 341-352). Phosphorylation of S346/347 blocks antibody binding. PMID: 24154522, 25451233.</p> <p>We recommend dephosphorylation of samples using lambda phosphatase treatment. Please refer to application notes.</p>
Tested applications	Suitable for: IHC-Fr, Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
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	<p>Our internal data indicates that mouse and rat are not recommended for IHC.</p> <p>Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).</p> <p>See other anti-rabbit secondary antibodies that can be used with this antibody.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	UMB2
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab124824 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	★★★★★ (3)	Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB	★★★★★ (9)	1/100. Predicted molecular weight: 39 kDa. Being CXCR4 a membrane protein, we recommend to not boil the samples after the lysis (before loading the samples on the WB gel). 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
IHC-P	★★★★★ (2)	1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols . 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	★★★★★ (3)	Use a concentration of 5 µg/ml.

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
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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.

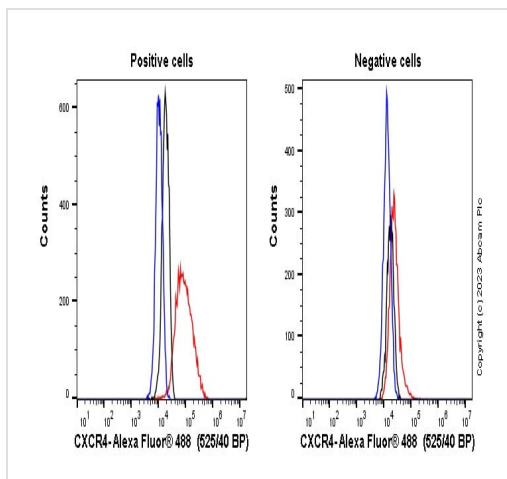
Sulfation on Tyr-21 is required for efficient binding of CXCL12/SDF-1alpha and promotes its dimerization.

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] (ab124824)

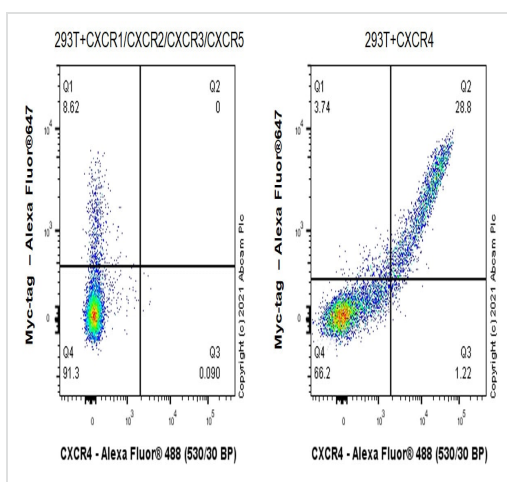
Flow cytometry overlay histogram showing left Jurkat positive cells and right negative HepG2 stained with ab124824 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat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

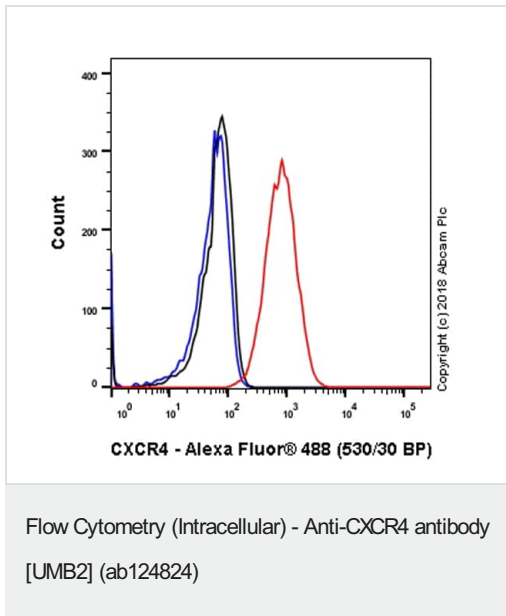
This antibody gave a positive signal in Jurkat Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



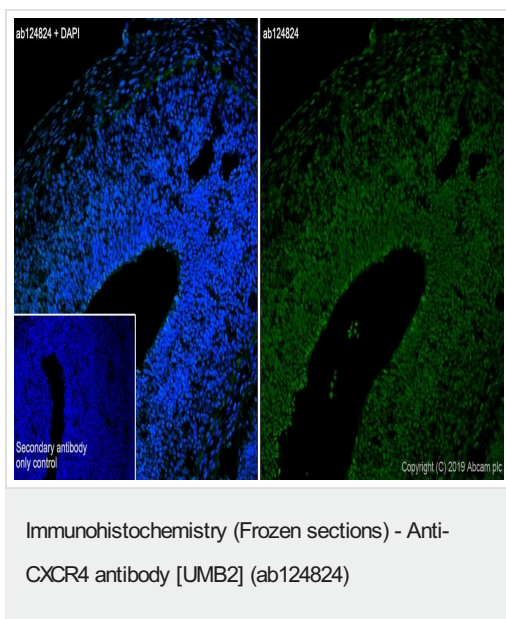
Flow Cytometry (Intracellular) - Anti-CXCR4 antibody
[UMB2] (ab124824)

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 (0.1 μ g)/ 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.

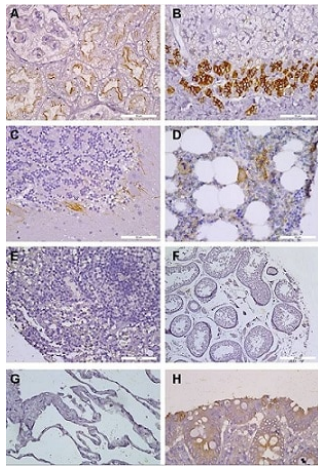


Intracellular Flow Cytometry analysis of Jurkat (human T cell leukemia T lymphocyte) cells labeling CXCR4 with purified ab124824 at 1/260 dilution (10 µg/ml) - Red. Cells were fixed with 4% paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) - Black. Unlabeled control - Blue. Untreated cells - Green



Immunohistochemical (Frozen) analysis of mouse E14.5 cerebrum labeling CXCR4 with ab124824 at 1/250 (10.8 µg/mL). Sections were fixed with 4% PFA and permeabilised with 0.2% Triton X-100. **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2 µg/mL) was used as the secondary antibody. Nuclei were counterstained blue with DAPI. Heat mediated antigen retrieval was performed using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

Positive staining on mouse embryonic cerebrum.



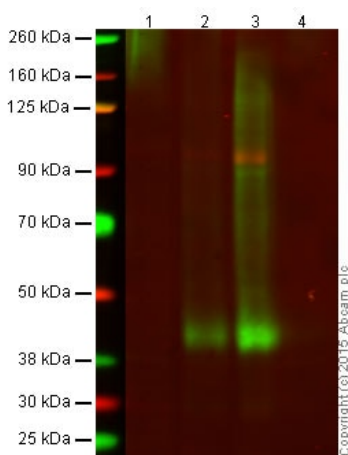
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] (ab124824)

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



Western blot - Anti-CXCR4 antibody [UMB2] (ab124824)

All lanes : Anti-CXCR4 antibody [UMB2] (ab124824)

Lane 1 : CHO (negative control)

Lane 2 : Jurkat whole cell

Lane 3 : Jurkat membrane

Lane 4 : Jurkat nuclear (negative control)

Lysates/proteins at 20 μ g per lane.

Secondary

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

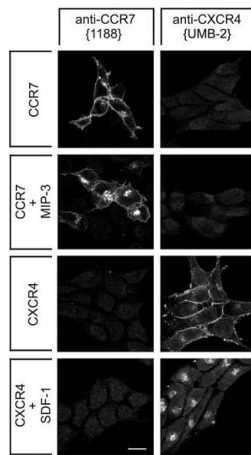
Predicted band size: 39 kDa

Observed band size: 41 kDa

Running buffer: MOPS.

Conditions: denatured/reduced.

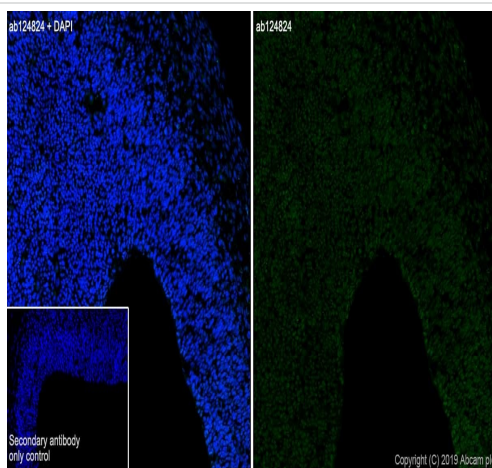
This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with ab124824 (anti-CXCR4) and **ab7671** (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.



Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [UMB2] (ab124824)

Fischer, T. et al PLoS One. 2008;3(12):e4069. doi: 10.1371/journal.pone.0004069. Epub 2008 Dec 31
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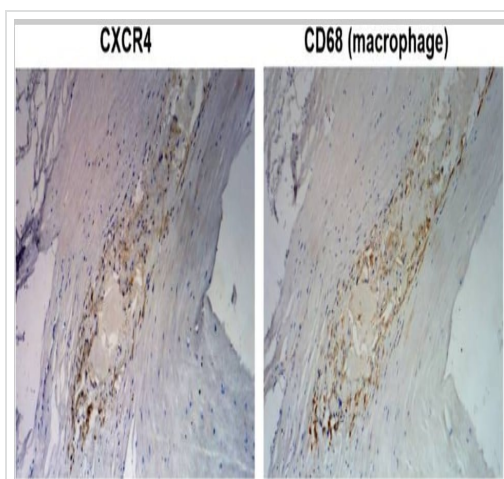
Characterization of UMB-2 (ab124824) by immunofluorescent staining of transfected cells. HEK-293 cells expressing CCR7 or CXCR4 were either not exposed or exposed to 100 ng/ml MIP-3 or 100 ng/ml SDF-1 for 30 min, subsequently fixed and immunofluorescently stained with 1 µg/ml anti-CCR7 {1188} or anti-CXCR4 {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.



Immunohistochemistry (Frozen sections) - Anti-CXCR4 antibody [UMB2] (ab124824)

Immunohistochemical (Frozen) analysis of rat E14.5 cerebrum labeling CXCR4 with ab124824 at 1/250 (10.8 µg/mL). Sections were fixed with 4% PFA and permeabilised with 0.2% Triton X-100. **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2 µg/mL) was used as the secondary antibody. Nuclei were counterstained blue with DAPI. Heat mediated antigen retrieval was performed using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

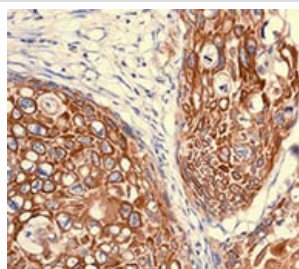
Positive staining on rat embryonic cerebrum.



Immunohistochemistry analysis of cryosections from carotid endarterectomy specimens labeling CXCR4 with ab124824 at 1/300 dilution. CXCR4 expression in a representative inflamed carotid plaque lesion. Brightfield micrographs showed brown chemoimmunoreactive CXCR4 and CD68 (macrophage) staining. Co-localized CXCR4 and CD68 expression was observed in these two adjacent sections.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] (ab124824)

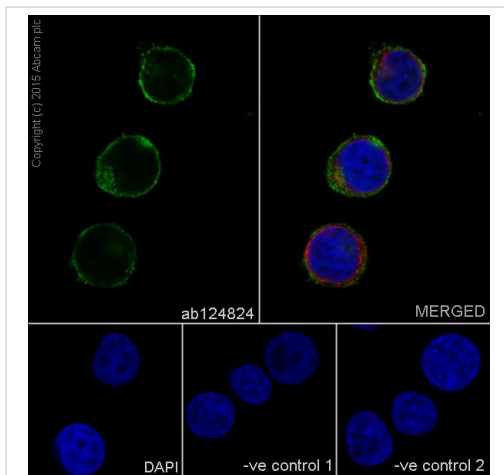
Li X et al., European journal of nuclear medicine and molecular imaging. 45,4 (2018): 558-566. Fig 3. doi:10.1007/s00259-017-3831-0. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>



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

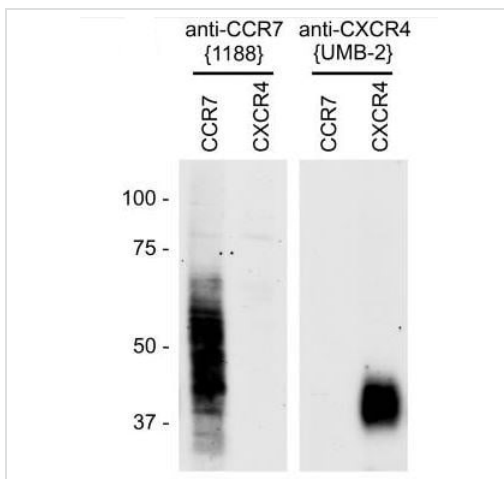
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] (ab124824)



Immunocytochemistry/ Immunofluorescence - Anti-CXCR4 antibody [UMB2] (ab124824)

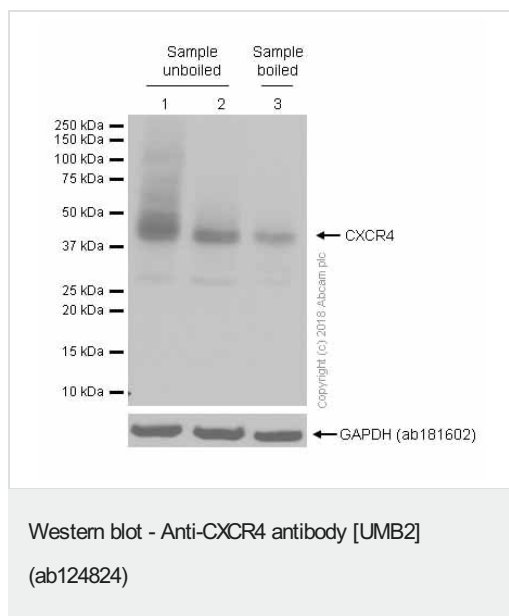
Immunofluorescence staining of Jurkat cells with purified ab124824 at a working dilution of 1 in 250, counter-stained with DAPI. Tubulin was stained with mouse anti-tubulin at a dilution of 1/1000 (**ab7291**) and Alexa Fluor® 594 goat anti-mouse at a dilution of 1/500 (**ab150120**). The secondary antibody 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.



Western blot - Anti-CXCR4 antibody [UMB2] (ab124824)

Western blot analysis of the specificity of anti-CXCR4 antibodies. Membrane preparations from HEK-293 cells stably transfected to express either CCR7 or CXCR4 were separated on 10% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes. Membranes were then incubated with affinity-purified 1 µg/ml anti-CCR7 {1188} or anti-CXCR4 {UMB-2} hybridoma supernatant at a dilution of 1:100. Blots were developed using enhanced chemiluminescence. Note that UMB-2 detected a band of the expected molecular weight only in CXCR4- but not in CCR7-transfected cells. Two additional experiments gave similar results.

Fischer, T. et al PLoS One. 2008;3(12):e4069. doi: 10.1371/journal.pone.0004069. Epub 2008 Dec 31. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>.



All lanes : Anti-CXCR4 antibody [UMB2] (ab124824) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lanes 2-3 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

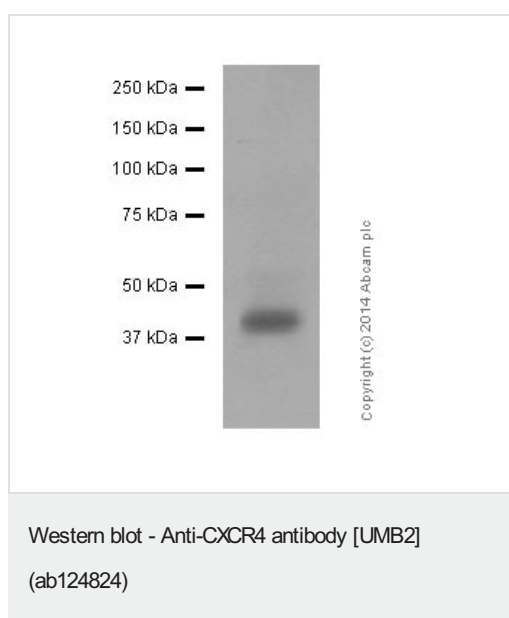
Predicted band size: 39 kDa

Observed band size: 43 kDa

Exposure time: 1 second

Blocking and diluting buffer: 5% NFDM/TBST

We suggest to not boil the sample after lysis.



Anti-CXCR4 antibody [UMB2] (ab124824) at 1/100 dilution (purified) + WI-38 cell lysate at 10 µg

Secondary

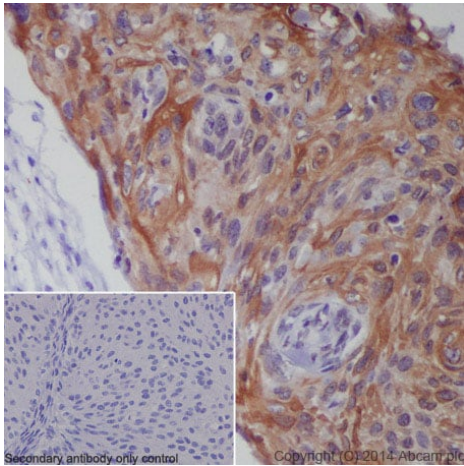
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 39 kDa

Observed band size: 43 kDa

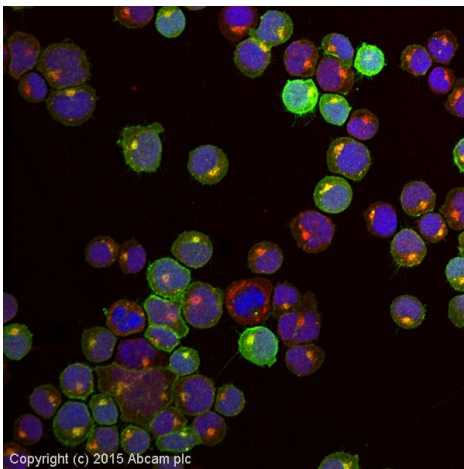
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



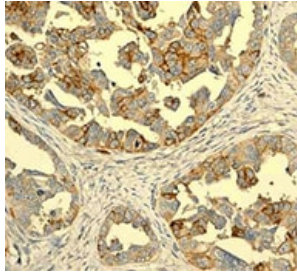
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXC4 antibody [UMB2] (ab124824)

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 performed 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.



Immunocytochemistry/ Immunofluorescence - Anti-CXC4 antibody [UMB2] (ab124824)

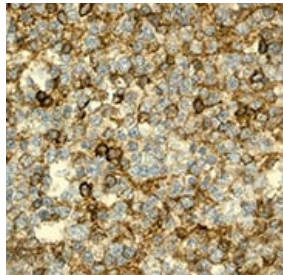
ab124824 stained Jurkat cells. The cells were 100% methanol fixed for 5 minutes at -20°C and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] (ab124824)

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

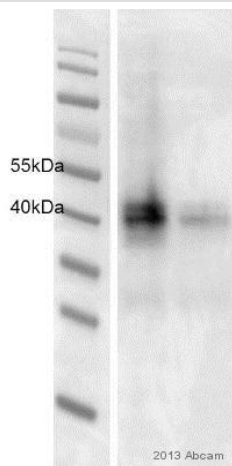
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CXCR4 antibody [UMB2] (ab124824)

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

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



Western blot - Anti-CXCR4 antibody [UMB2] (ab124824)

This image is courtesy of an anonymous Abreview

Lane 1 : Anti-CXCR4 antibody [UMB2] (ab124824) at 1/500 dilution (unpurified)

Lane 2 : Anti-CXCR4 antibody [UMB2] (ab124824) at 1/500 dilution

Lane 1 : HEK239 transfected with a CXCR4 (mouse) expression vector cell lysate

Lane 2 : HEK239 transfected with an empty expression vector cell lysate

Lysates/proteins at 100000 cells per lane.

Secondary

All lanes : HRP-conjugated Goat anti-rabbit IgG polyclonal at 1/50000 dilution

Developed using the ECL technique.





Performed under reducing conditions.

Predicted band size: 39 kDa

Observed band size: 42,47 kDa

Exposure time: 10 seconds

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-CXCR4 antibody [UMB2] (ab124824)

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

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