BOSTER BIOLOGICAL TECHNOLOGY
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Anti-CXCR4 Antibody

Catalog Number: A00031-1

About CXCR4

CXCR4, a G-protein coupled receptor (GPCR) with seven transmembrane domains, is a CXC chemokine receptor specific for stromal-derived-factor-1 (SDF-1 or CXCL12). CXCR4 was initially discovered as one of the co-receptors for HIV entry into CD4+ T cells (1). Blocking CXCR4 could be potentially used as novel therapeutics for HIV treatment.

CXCR4 signaling plays an important role in the migration, proliferation and quiescence of hematopoietic stem cell and their retention within the bone marrow, where it has high levels of SDF-1/CXCL12(2). It has been demonstrated that CXCR4 signaling mediates CD20 up-regulation on B cells (3).

CXCR4 is highly expressed in more than 23 types of cancer, including breast cancer, ovarian cancer, melanoma, and prostate cancer, while there is very less or no expression of CXCR4 in healthy tissues. CXCR4 expression in cancer cells has been reported to be associated with tumor survival, growth and metastasis in tissues with high levels of SDF-1/CXCL12, such as lungs, liver and bone marrow (4,5).

CXCR4 has been shown to regulate neuronal migration, cell positioning and axon wiring (6,7). CXCR4 mutant mice displayed aberrant neuronal distribution, which implicates the role in neuronal disorders such as epilepsy. CXCR4 is also involved in WHIM syndrome (8). WHIM mutations in CXCR4 were recently found in patients with Waldenstrom's macroglobulinemia, and these mutations are correlated to clinical resistance to ibrutinib (9,10).

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Overview

Product Name	Anti-CXCR4 Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-CXCR4 Antibody (Catalog # A00031-1). Tested in ELISA, WB, ICC, IF, Flow Cytometry, IHC-P applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC-P, ICC, WB
Clonality	Polyclonal
Formulation	CXCR4 Antibody is supplied in PBS containing ≤0.09% sodium azide.
Storage Instructions	CXCR4 antibody can be stored at 4°C, stable for up to one year. Avoid repeated freeze-thaw cycles. Antibodies should not be exposed to prolonged high temperatures.

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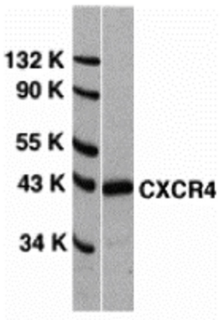
Host	Rabbit
Uniprot ID	P61073

Technical Details

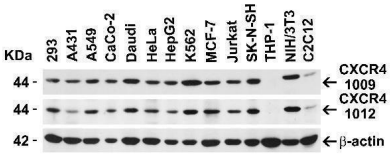
Immunogen	Anti-CXCR4 antibody was raised against a peptide corresponding to 14 amino acids near the amino terminus of human CXCR4 isoform b. The immunogen is located within the first 50 amino acids of CXCR4.
Predicted Reactive Species	Bovine, Pig
Cross Reactivity	CXCR4 Antibody is predicted to not cross-react with other CXCR familiy members.
Isotype	IgG
Form	Liquid
Concentration	1 mg/mL
Purification	CXCR4 Antibody is affinity chromatography purified via peptide column.
Suggested Dilutions	WB: 1 - 2 ug/mL; IP/ ICC: 2 ug/mL; IHC-P: 5 ug/mL; IF: 20 ug/mL; Flow Cyt: 0.1 ug/mL. Antibody validated: Western Blot in human, mouse, and rat samples; Immunohistochemistry, Immunocytochemistry and Immunofluorescence in human samples; Flow Cytometry in human and mouse samples. All other applications and species not yet tested. Optimal dilutions for each application should be determined by the researcher.

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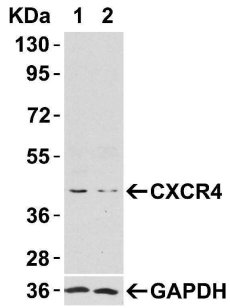
Anti-CXCR4 Antibody (A00031-1) Images



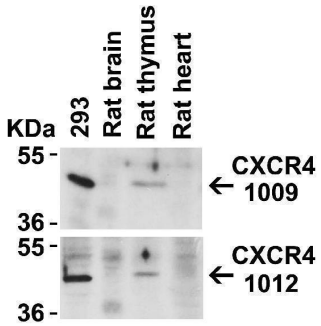
Western Blot Validation of CXCR4 in HeLa Cells Loading: 15 ug of lysates per lane. Antibodies: A00031-1 (1 ug/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



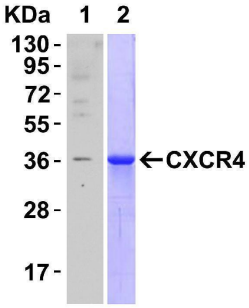
Independent Antibody Validation (IAV) via Protein Expression Profile in Cell Lines Loading: 15 ug of lysates per lane. Antibodies: A00031-1 (1 ug/mL), 1012 (1 ug/mL), and beta-actin (1 ug/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



Validation with CXCR4 siRNA Knockdown in HeLa Cells HeLa cells were transfected with control siRNAs (lane 1) or CXCR4 siRNAs (lane 2) Loading: 10 ug of HeLa whole cell lysates per lane. Antibodies: A00031-1 (2 ug/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

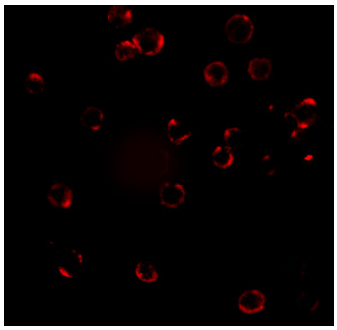


Animal Species Reactivity Loading: Lysates/proteins at 20 ug per lane. Antibodies: A00031-1 (2 ug/mL) or 1012 (2 ug/mL). 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

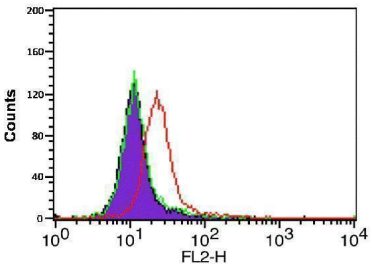


Recombinant Protein Test Loading: CXCR4 partial recombinant protein (Novus Biologicals, Cat# H00007852-Q01). Lane 1: Anti-CXCR4 antibody (0.1 ug/mL) 1 h incubation at RT in 5% NFDM/TBST. Lane 2: Coomassie blue staining. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

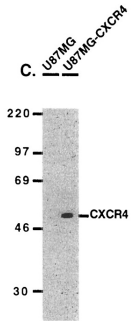
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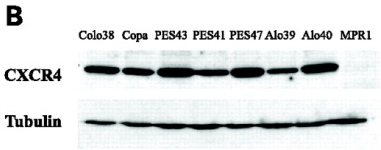
Immunofluorescence Validation of CXCR4 in HeLa Cells Immunofluorescent analysis of 4% paraformaldehyde-fixed HeLa cells labeling CXCR4 with A00031-1 at 20 ug/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red). Image showing both membrane and cytoplasmic staining on HeLa cells.



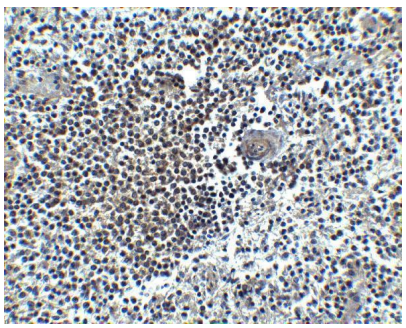
Flow Cytometry Validation of CXCR4 in HeLa Cells Overlay histogram showing HeLa cells stained with A00031-1 (red line, 1ug/1x106 cells). 1 h incubation at 4˚C in 2% FBS/PBS. Followed by secondary antibody 488 goat anti-rabbit IgG (H+L) at 1/500 dilution for 1 h 4˚C. Isotype control antibody (Green line) was mouse IgG1 (1ug/1x106 cells) used under the same conditions.



Overexpression Validation of CXCR4 (Kozak et al., 2002) U87MG and U87MG-CXCR4 extracts were included as negative and positive controls, respectively, for CXCR4 detection with anti-CXCR4 antibodies.



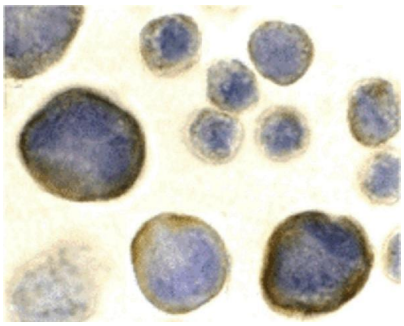
WB Validation of CXCR4 in Human Metastatic Melanoma (Scala et al., 2006) CXCR4 protein was detected in the human metastatic melanoma cell lines and human melanoma cell line (colo38), but not in the human primary melanocytes (MPR1) with anti-CXCR4 antibodies.



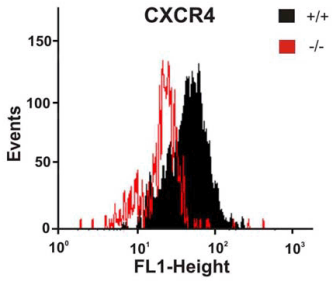
Immunohistochemistry Validation of CXCR4 in Human Spleen Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-CXCR4 antibody (A00031-1) at 5 ug/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4˚C. A Goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.

Immunocytochemistry Validation of CXCR4 in HeLa Cells Immunocytochemical analysis of HeLa cells using anti-CXCR4 antibody (A00031-1) at 2 ug/ml. Cells was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer

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(pH6). Samples were incubated with primary antibody overnight at 4˚C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.



KO Validation of CXCR4 by Flow Cytometry (?demis, et al., 2010) Astrocytes from wild-type or CXCR4 knockout mice were stained with primary antibodies against CXCR4 and FITC-labeled secondary antibodies, and subsequently subjected to flow cytometry. CXCR4?/? astrocytes (red) showed loss of CXCR4 cell-surface expression compared with wild-type cells (black).

3 Publications Citing This Product

1. PubMed ID: 33413641, Gao W,Yang X,Du J,Wang H,Zhong H,Jiang J,Yang C.Glucocorticoid guides mobilization of bone marrow stem/progenitor cells via FPR and CXCR4 coupling.Stem Cell Res Ther.2021 Jan 7;12(1):16.doi:10.1186/s13287-020-02071-1.PMID:33413641;PMCID:PMC7791823.

2. PubMed ID: 18983683, Chemokine CXCL12 and its receptor CXCR4 expression are associated with perineural invasion of prostate cancer

3. PubMed ID: 24912495, Tacrolimus promotes hepatocellular carcinoma and enhances CXCR4/SDF-1? expression?in vivo

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Anti-CXCR4 Antibody

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