Product Datasheet

CXCR4 Antibody
NB100-74396

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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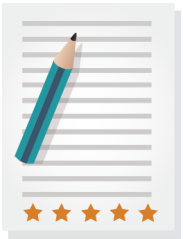
Updated 10/23/2024 v.20.1



NB100-74396

CXCR4 Antibody

Product Information

Unit Size	0.1 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Unpurified
Buffer	Whole antisera

Product Description

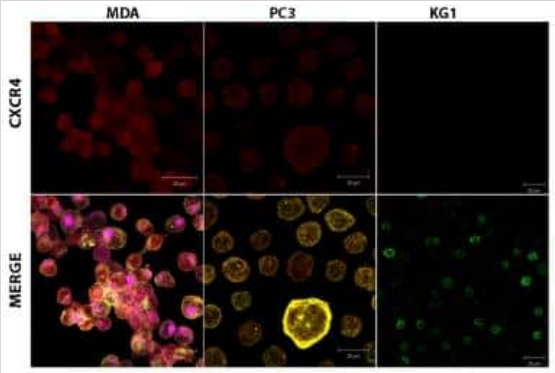
Host	Rabbit
Gene ID	7852
Gene Symbol	CXCR4
Species	Human, Mouse, Rat
Reactivity Notes	Rat reactivity reported in scientific literature (PMID:33116799).
Immunogen	A synthetic peptide made to a C-terminal region of the human CXCR4 protein (within residues 300-352). [Swiss-Prot P61073]

Product Application Details

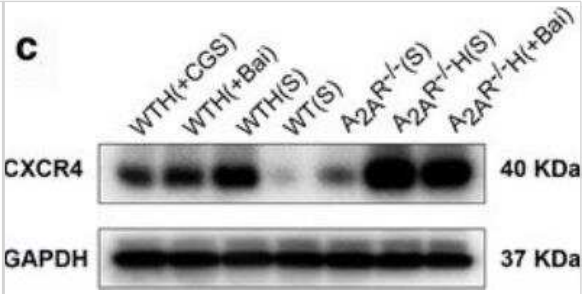
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:5000, Flow Cytometry, Immunohistochemistry 1:200-1:1000, Immunocytochemistry/ Immunofluorescence 1:50, Immunohistochemistry-Paraffin 1:200-1:1000, Immunohistochemistry-Frozen

Images

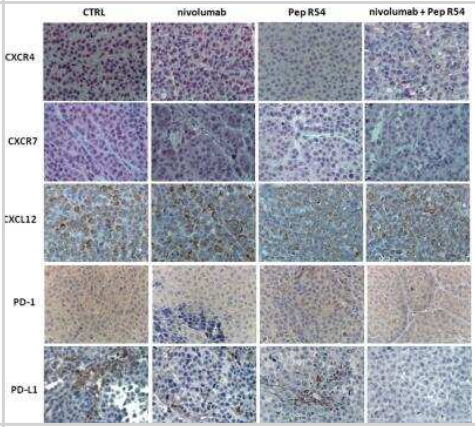
Immunocytochemistry/Immunofluorescence: CXCR4 Antibody [NB100-74396] - Optimization of immunofluorescence staining of CXCR4 protein. MDA, PC3, KG1 cells were used for the optimization experiments. Cells were seeded onto cell-tak coated 48 mm coverslips in a 48-well plate. Cells were fixed with 2% formaldehyde for 20 min, washed with PBS. After fixation, cells were blocked with 2.0% BSA in PBS for 1 h at room temperature. All the cells were incubated with a primary antibody for anti-rabbit CXCR4 for 1 h. After washing with PBS, cells were put in respective secondary antibodies-anti-rabbit dylight 405 for 1 h. PSMA= Magenta, EpCAM= Yellow, sLex= Green, CXCR4= Red, and Merge shows all the colors. MDA=PSMA+, EpCAM+, sLex+, CXCR4+. PC3 = PSMA-, EpCAM+, sLex-, CXCR4+. KG1 = PSMA-, EpCAM-, sLex+, CXCR4-. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0085143) licensed under a CC-BY license.



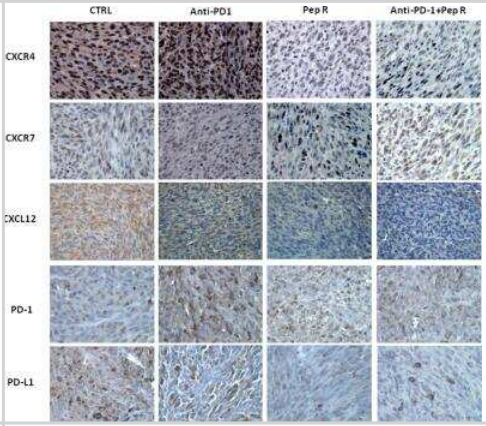
Western Blot: CXCR4 Antibody [NB100-74396] - The A2AR and baicalin attenuated CXCR4 expression in the hypoxia-induced PAH mouse model. CXCR4 protein expression levels in lung tissue were examined by western blot. GAPDH served as an internal control (c, d; n = 3). Image collected and cropped by CiteAb from the following publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5543745/) licensed under a CC-BY license.



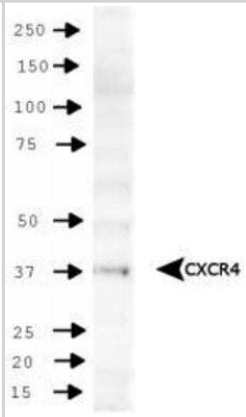
Immunohistochemistry: CXCR4 Antibody [NB100-74396] - Pep R54 in combination with nivolumab reduced the expression of CXCR4-CXCL12 and PD-L1 in PES43 tumors. Representative IHC pictures (magnification 400x) for CXCR4, CXCR7 (red staining), CXCL12, PD-1 and PD-L1 expression (brown staining) in PES43 collected tumors from mice treated with Pep R54, nivolumab or combined treatment. Image collected and cropped by CiteAb from the following publication (https://jeccr.biomedcentral.com/articles/10.1186/s13046-019-1420-8) licensed under a CC-BY license.



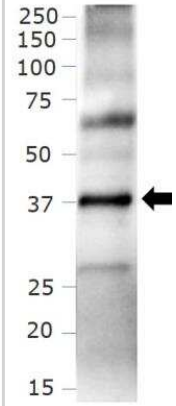
Immunohistochemistry: CXCR4 Antibody [NB100-74396] - Pep R in combination with anti-PD-1 reduced the expression of CXCR4-CXCL12 and PD-L1 in MC38 tumors. Representative IHC pictures for CXCR4, CXCR7, CXCL12, PD-1 and PD-L1 expression (brown staining) in MC38 collected tumors (magnification 400x), from mice treated with Pep R, anti-murine PD-1 or combined treatment showing the reduction of CXCR4, CXCL12 and PD-L1 expression in mice treated with Pep R alone and in combination with anti-PD-1Image collected and cropped by CiteAb from the following publication (https://jeccr.biomedcentral.com/articles/10.1186/s13046-019-1420-8) licensed under a CC-BY license.



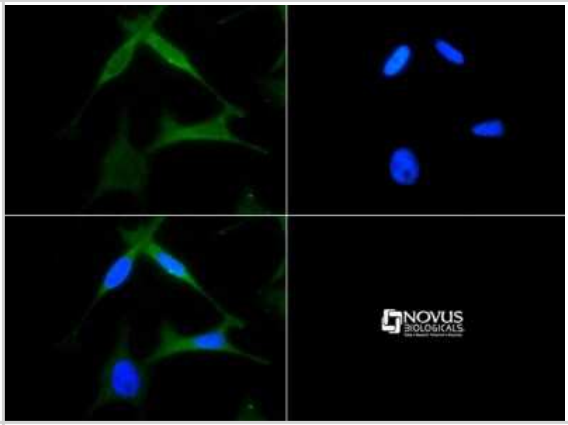
Western Blot: CXCR4 Antibody [NB100-74396] - Analysis of CXCR4 in HeLa whole cell extract.



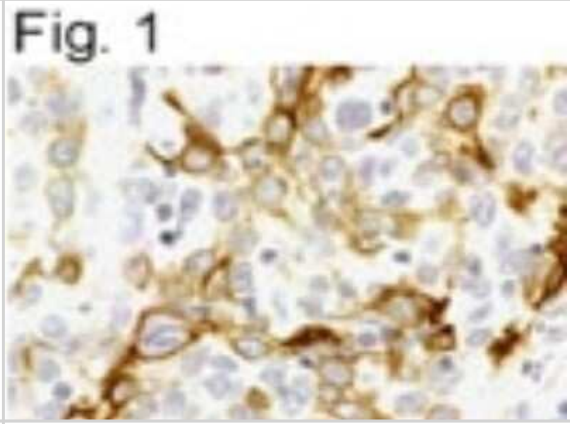
Western Blot: CXCR4 Antibody [NB100-74396] - Analysis of CXCR4 protein in human small intestine tissue lysate using 1:500 dilution of rabbit polyclonal CXCR4 antibody (Lot A2). The signal was developed using ECL method and the antibody generated a specific band of CXCR4 at ~40 kDa position.



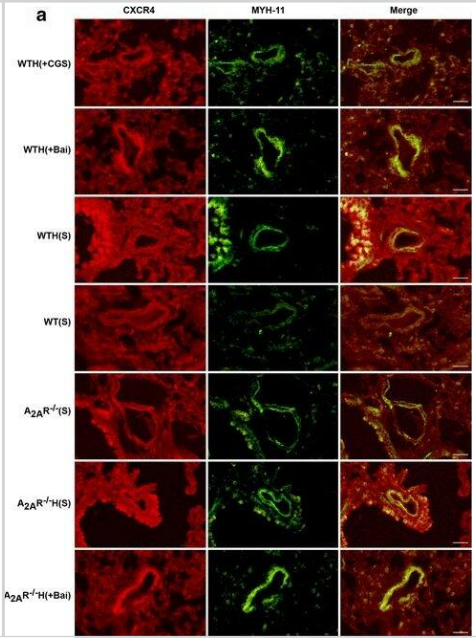
Immunocytochemistry/Immunofluorescence: CXCR4 Antibody [NB100-74396] - CXCR4 antibody was tested in HeLa cells with FITC (green). Nuclei were counterstained with DAPI (blue).



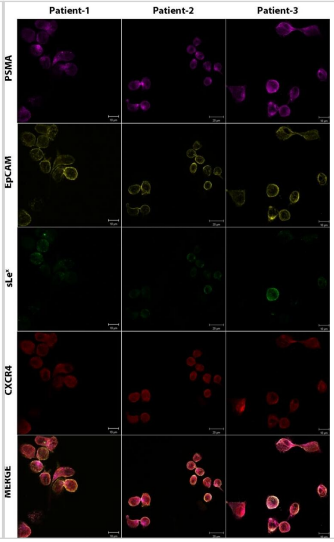
Immunohistochemistry: CXCR4 Antibody [NB100-74396] - Immunostaining of CXCR4 in human cervical carcinoma tissue sections.



Immunocytochemistry/ Immunofluorescence: CXCR4 Antibody [NB100-74396] - The A2AR & baicalin attenuated CXCR4 expression in the hypoxia-induced PAH mouse model. CXCR4 & MYH11 expression levels in mouse PASMCs were analyzed by immunofluorescence staining (a; n = 3). CXCR4 protein is stained red, & MYH11 is stained green to indicate the PASMCs (×400; scale bars indicate 50 μm). CXCR4 fluorescence intensity was calculated (b; n = 3). CXCR4 protein expression levels in lung tissue were examined by western blot. GAPDH served as an internal control (c, d; n = 3). Data are presented as the mean ± SD. #Value significantly greater than the corresponding value in saline-treated normoxic mice (##P < 0.01). *Value significantly less than the corresponding value in hypoxic mice (*P < 0.05, **P < 0.01). §Value significantly less than the corresponding value in baicalin-treated mice (§P < 0.05, §§P < 0.01). +Value significantly different between WT & A2AR−/− mice (+P < 0.05, ++P < 0.01). WTH, wild-type hypoxic; A2AR−/−H, A2AR−/− hypoxic; s, saline-treated Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28774332), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: CXCR4 Antibody [NB100-74396] - Isolation of prostate CTCs from metastatic PCa patients using anti-CD45 immunomagnetic depletion.2.5 ml blood from three metastatic PCa patients (> 50 CTCs/ 2.5 ml blood) was processed via ficoll density centrifugation & the PBMC fraction was collected. Immunomagnetic anti-CD45 depletion was performed on the obtained PBMCs & the remaining cells were washed, cytospunned onto the slides. Slides were stained for PSMA, EpCAM, sLex, & CXCR4 using the protocol as described in Figure S1. MDA, PC3, & KG1 cells were simultaneously stained as a control for the following markers: PSMA= Magenta, EpCAM= Yellow, HECA-452= Green, CXCR4= Red. All prostate CTCs expressed CXCR4, while, sLex expression was variable. The analysis of sLex intensity is shown in Figure 4. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24386459), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Filidou E, Kandilogiannakis L, Tarapatzi G et al. A Simplified and Effective Approach for the Isolation of Small Pluripotent Stem Cells Derived from Human Peripheral Blood Biomedicines 2023-03-05 [PMID: 36979766] (Immunocytochemistry/ Immunofluorescence, Human)

Ranjith Palanisamy, Nimnaka Indrajith Kahingalage, David Archibald, Ilaria Casari, Marco Falasca Synergistic Anticancer Activity of Plumbagin and Xanthohumol Combination on Pancreatic Cancer Models. International journal of molecular sciences 2024-02-26 [PMID: 38397018]

Wang Y, Liu Y, Li XY et al. Vagus nerve stimulation-induced stromal cell-derived factor-l alpha participates in angiogenesis and repair of infarcted hearts ESC heart failure 2023-08-29 [PMID: 37641543] (ICC/IF, Human)

Zhang S, Peng B, Chen Z et al. Brain-targeting, acid-responsive antioxidant nanoparticles for stroke treatment and drug delivery Bioactive Materials 2022-10-01 [PMID: 35386312]

Wu, H;Peng, B;Mohammed, FS;Gao, X;Qin, Z;Sheth, KN;Zhou, J;Jiang, Z; Brain Targeting, Antioxidant Polymeric Nanoparticles for Stroke Drug Delivery and Therapy Small (Weinheim an der Bergstrasse, Germany) [PMID: 35306743] (WB, Mouse)

Tagami M, Kakehashi A, Katsuyama-Yoshikawa A et al. FOXP3 and CXCR4-positive regulatory T cells in the tumor stroma as indicators of tumor immunity in the conjunctival squamous cell carcinoma microenvironment PLOS ONE 2022-03-31 [PMID: 35358193] (IF/IHC, Human)

Zhang X, Detering L, Sultan D et al. C-X-C Chemokine Receptor Type 4-Targeted Imaging in Glioblastoma Multiforme Using 64Cu-Radiolabeled Ultrasmall Gold Nanoclusters ACS Applied Bio Materials 2021-12-23 [PMID: 35014818] (IHC-Fr)

Zhang, S, Deng, G Et al. Autocatalytic Delivery of Brain Tumor-targeting, Size-shrinkable Nanoparticles for Treatment of Breast Cancer Brain Metastases. Adv Funct Mater 2020-04-03 [PMID: 32440263] (ELISA, Rat)

Guan W, Li F, Zhao Z et al. Tumor-Associated Macrophage Promotes the Survival of Cancer Cells upon Docetaxel Chemotherapy via the CSF1/CSF1R-CXCL12/CXCR4 Axis in Castration-Resistant Prostate Cancer Genes 2021-05-19 [PMID: 34069563] (WB, Mouse)

D'Alterio C, Buoncervello M et al. Targeting CXCR4 potentiates anti-PD-1 efficacy modifying the tumor microenvironment and inhibiting neoplastic PD-1. J Exp Clin Cancer Res 2019-10-28 [PMID: 31661001] (IF/IHC, Mouse)

Peng C, Chen XT, Xu H et al. Role of the CXCR4/ALK5/Smad3 Signaling Pathway in Cancer-Induced Bone Pain J Pain Res 2020-10-14 [PMID: 33116799] (WB, Rat)

Zhou Y, Zhang S, Chen Z et al. Targeted Delivery of Secretory Promelittin via Novel Poly(lactone co-beta amino ester) Nanoparticles for Treatment of Breast Cancer Brain Metastases Adv. Sci. 2020-01-19 [PMID: 32154067] (IF/IHC, FLOW, Human)

More publications at http://www.novusbio.com/NB100-74396

Procedures

Western Blot protocol for CXCR4 Antibody (NB100-74396)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room

temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NB100-74396

NB800-PC1	HeLa Whole Cell Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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