

# Product Datasheet

## CCR2 Antibody - BSA Free

### NBP1-48338

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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Updated 10/23/2024 v.20.1

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**NBP1-48338**

CCR2 Antibody - BSA Free

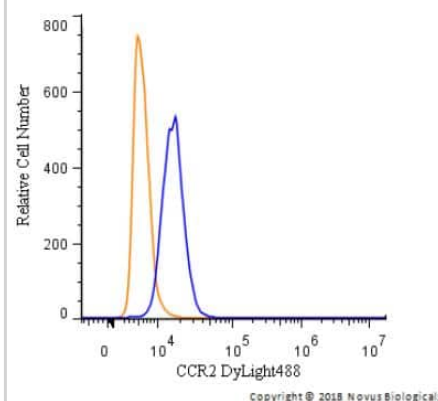
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	729230
Gene Symbol	CCR2
Species	Human, Mouse
Immunogen	Synthetic peptide made to an N-terminal portion of the mouse CCR2 protein (within residues 20-100). [Swiss-Prot# P51683]

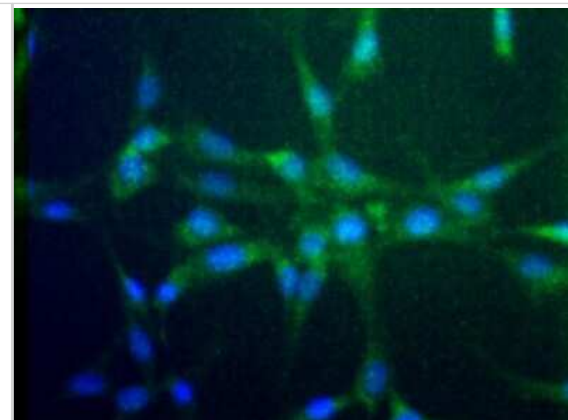
Product Application Details	
Applications	Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen
Recommended Dilutions	Flow Cytometry, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:50, Immunohistochemistry-Frozen reported in scientific literature (PMID 31399604)

**Images**

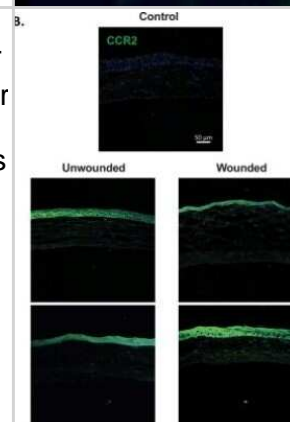
Flow Cytometry: CCR2 Antibody [NBP1-48338] - An intracellular stain was performed on THP-1 cells with CCR2 Antibody NBP1-48338G (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 488.



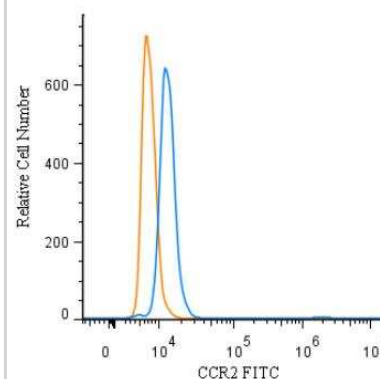
Immunocytochemistry/Immunofluorescence: CCR2 Antibody [NBP1-48338] - Analysis of CCR2 in NIH/3T3 cells.



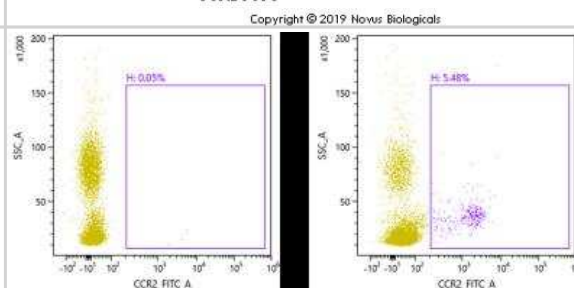
Immunohistochemistry: CCR2 Antibody [NBP1-48338] - Expression patterns of CCL2 and CCR2 in unwounded and wounded corneas of WT and MMP12 KO mice. Immunofluorescence of CCL2 chemokine receptor CCR2 in unwounded and chemically wounded (2-days after injury) WT and Mmp12<sup>-/-</sup> mouse corneas. Control images represent mouse corneas stained with secondary antibody only and without primary antibody. Nuclei were visualized by staining with DAPI (blue). Scale bars: 50  $\mu$ m. CCR2 staining was visualized in epithelial and stromal layers of unwounded and wounded WT and Mmp12<sup>-/-</sup> corneas. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-019-47831-z>), licensed under a CC-BY license.



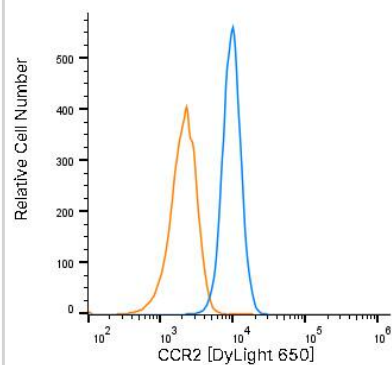
Flow Cytometry: CCR2 Antibody [NBP1-48338] - An intracellular stain was performed on THP-1 cells with CCR2 Antibody NBP1-48338F (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 10  $\mu$ g/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.



Flow Cytometry: CCR2 Antibody [NBP1-48338] - The Alexa Fluor 488 conjugate of this antibody was used: Mouse peripheral blood mononuclear cells were unstained (left) or stained (right) with CCR2 antibody. Image from verified customer review.

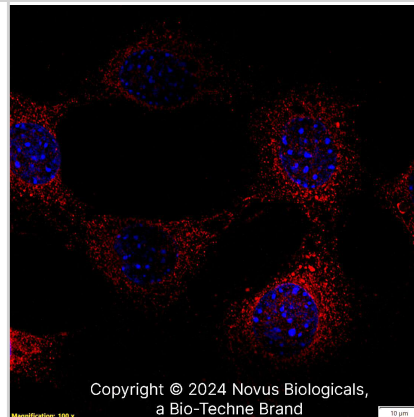


THP-1 human acute monocytic leukemia cell line was stained with Rabbit anti-CCR2 Affinity-purified Polyclonal Antibody conjugated to DyLight 650 (Catalog # NBP1-48338C, blue histogram) or matched control antibody (Catalog #NBP2-24891C, orange histogram).



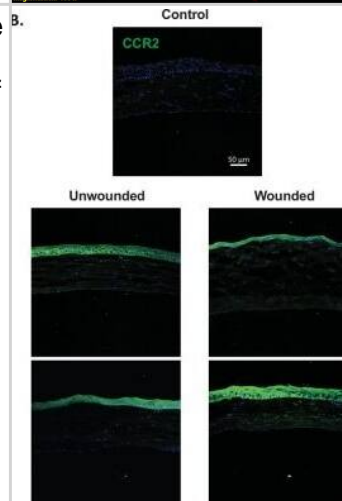
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CCR2 was detected in immersion fixed NIH3T3 Mouse fibroblast cell line using Rabbit anti-CCR2 Antigen Affinity-purified Polyclonal Antibody conjugated to DyLight 550 (Catalog # NBP1-48338R) (red) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



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Immunocytochemistry/ Immunofluorescence: CCR2 Antibody - BSA Free [NBP1-48338] - Expression patterns of CCL2 & CCR2 in unwounded & wounded corneas of WT & MMP12 KO mice. (A) Immunofluorescence of CCL2 chemokine & (B) its receptor CCR2 in unwounded & chemically wounded (2-days after injury) WT & Mmp12<sup>-/-</sup> mouse corneas. Control images represent mouse corneas stained with secondary antibody only & without primary antibody. Nuclei were visualized by staining with DAPI (blue). Scale bars: 50 µm. A magnified image of a wounded WT cornea shows perinuclear expression of CCL2 (orange box). CCL2 staining was visualized in epithelial, stromal, & endothelial layers of wounded WT & Mmp12<sup>-/-</sup> corneas. CCR2 staining was visualized in epithelial & stromal layers of unwounded & wounded WT & Mmp12<sup>-/-</sup> corneas. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31399604>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Elizondo-Benedetto S, Sastriques-Dunlop S, Detering L et al. Chemokine Receptor 2 Is A Theranostic Biomarker for Abdominal Aortic Aneurysms medRxiv : the preprint server for health sciences 2023-11-07 [PMID: 37986880] (IHC-P, Rat)

Deng S, Zhou F, Wang F et al. C5a enhances V $\beta$ 1 T cells recruitment via the CCL2-CCR2 axis in IgA nephropathy International immunopharmacology 2023-10-18 [PMID: 37862725] (IHC-P, Human)

Details:  
HMC Stimulation

Balkas DA, Datz S, Meyer-Schwickerath C et al. Organ-restricted vascular delivery of nanoparticles for lung cancer therapy Advanced Therapeutics 2021-04-22 [PMID: 33884290] (Immunocytochemistry/ Immunofluorescence)

Kim S, Oh D, Choi H et al. The effect of C-C motif chemokine ligand 2 supplementation on in vitro maturation of porcine cumulus-oocyte complexes and subsequent developmental competence after parthenogenetic activation Frontiers in veterinary science 2023-03-13 [PMID: 36992978] (Immunocytochemistry/ Immunofluorescence, Porcine)

Chakrabarti J, Dua-Awereh M, Schumacher M et al. Sonic Hedgehog acts as a macrophage chemoattractant during regeneration of the gastric epithelium NPJ Regenerative medicine 2022-01-12 [PMID: 35022438] (IF/IHC, Mouse)

Lee SA, Kim D, Min C Et al. Phagocyte Chemoattraction Is Induced through the Mcp-1-Ccr2 Axis during Efferocytosis Cells 2021-11-10 [PMID: 34831339] (B/N, Human)

Baba T, Miyazaki D, Inata K et al. Role of IL-4 in bone marrow driven dysregulated angiogenesis and age-related macular degeneration Elife 2020-05-05 [PMID: 32366355] (IF/IHC, Mouse)

Details:  
Citation using the DyLight 550 format of this antibody.

Wolf M, Clay S. M, et al. MMP12 Inhibits Corneal Neovascularization and Inflammation through Regulation of CCL2. Sci Rep 2019-08-09 [PMID: 31399604] (IHC-Fr, Mouse)

Lang SC, Harre U, Purohit P et al. Neurodegeneration Enhances the Development of Arthritis. J. Immunol. 2017-02-10 [PMID: 28188247] (FLOW, Mouse)

Zhang L, Zhang S, Yao J et al. Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth. Nature 2015-11-05 [PMID: 26479035]

Carlson S, Helterline D, Asbe L et al. Cardiac macrophages adopt profibrotic/M2 phenotype in infarcted hearts: Role of urokinase plasminogen activator. J Mol Cell Cardiol 2016-06-01 [PMID: 27262672] (FLOW, Mouse)



## Procedures

### Flow (Intracellular) Protocol for CCR2 Antibody (NBP1-48338)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between  $2 \times 10^5$  and  $1 \times 10^6$  cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100  $\mu$ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
  - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of  $1 \times 10^6$  cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100  $\mu$ L samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100  $\mu$ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100  $\mu$ L of a permeabilization buffer to every  $1 \times 10^6$  cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
  - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
  - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100  $\mu$ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1  $\mu$ g per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200  $\mu$ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200  $\mu$ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500  $\mu$ L per sample) and proceed with analysis on your flow cytometer.

**Immunohistochemistry-Paraffin Protocol for CCR2 Antibody (NBP1-48338)**

## 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 (keep slides in the sodium citrate buffer at all times).

## Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) 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 HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.







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### **Products Related to NBP1-48338**

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NB800-PC8	NIH 3T3 Whole Cell Lysate
NBP1-48338PEP	CCR2 Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **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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