

# Product Datasheet

## CCR1 Antibody - BSA Free NB100-56334

Unit Size: 0.1 mg

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

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### Publications: 8

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**NB100-56334**

CCR1 Antibody - BSA Free

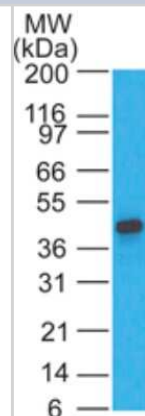
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	1230
Gene Symbol	CCR1
Species	Human, Mouse, Rabbit
Reactivity Notes	Rabbit reactivity reported in scientific literature (PMID: 20371596).
Immunogen	This antibody was raised against a synthetic peptide of human CCR1 protein.

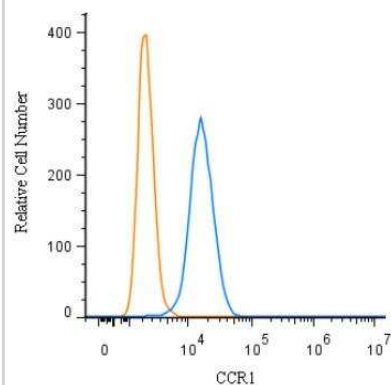
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen
Recommended Dilutions	Western Blot 1-3 ug/ml, Flow Cytometry 5-10 ul/test, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1 ug/ml, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen reported in scientific literature (PMID 15860797), Flow (Intracellular) reported in scientific literature (PMID 20371596)
Application Notes	In A375, a 42 kDa band should be observed.

**Images**

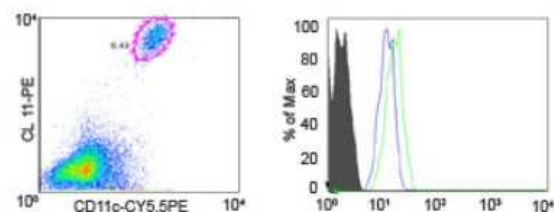
Western Blot: CCR1 Antibody [NB100-56334] - A375 lysate probed with this antibody at 2 ug/ml dilution.



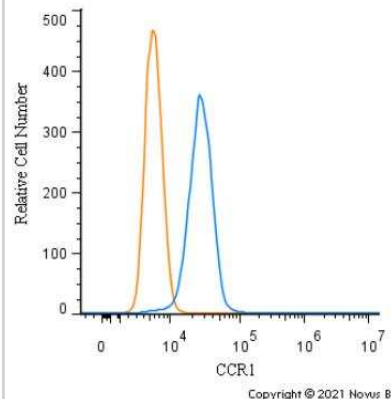
Flow (Intracellular): CCR1 Antibody [NB100-56334] - An intracellular stain was performed on Raw264.7 cells with CCR1 Antibody NB100-56334 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).



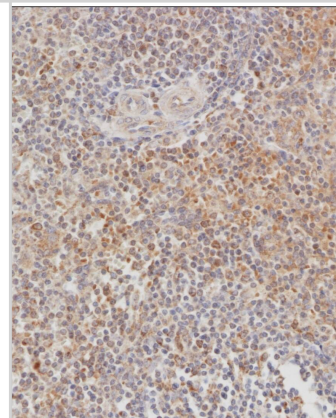
Flow Cytometry: CCR1 Antibody [NB100-56334] - Image 1: Surface staining of mouse primary epidermal cells (B-6 mice, 30wks) with CL 11-PE, CD11c PE-Cy5, and this antibody with CY5 goat anti rabbit secondary. Image 2: CD11c+ CL II+ cells (Langerans), stained with rabbit isotype control (shaded histogram) were analyzed for CCR1 at 5 ul (blue histogram) and 10 ul (green histogram). Data kindly provided by Corey Parlet, University of Iowa.



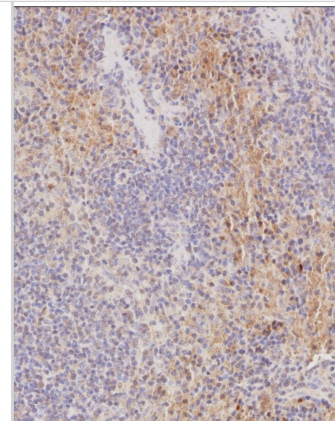
Flow (Intracellular): CCR1 Antibody [NB100-56334] - An intracellular stain was performed on U-87 cells with CCR1 Antibody NB100-56334 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).



Analysis of a FFPE tissue section of human spleen using 1:200 dilution of CCR1 antibody (NB100-56334). The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



Analysis of a FFPE tissue section of mouse spleen using 1:200 dilution of CCR1 (NB100-56334) antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



## Publications

Madi A, Sheinin R, Salomon K et al. interFLOW: maximum flow framework for the identification of factors mediating the signaling convergence of multiple receptors Research Square 2023-10-31 (IHC, Mouse)

Messex J, Adams K, Hawkins W et al. Oncogenic Kras-Mediated Cytokine CCL15 Regulates Pancreatic Cancer Cell Migration and Invasion through ROS Cancers 2022-04-26 [PMID: 35565279] (IHC-P, Human)

Shin SY, Lee DH, Lee J et al. C-C motif chemokine receptor 1 (CCR1) is a target of the EGF-AKT-mTOR-STAT3 signaling axis in breast cancer cells. Oncotarget 2017-11-07 [PMID: 29212252] (IF/IHC, Human)

Soehnlein Oliver, Drechsler Maik, Doring Yvonne et al. Distinct functions of chemokine receptor axes in the atherogenic mobilization and recruitment of classical monocytes. EMBO Mol Med. 2013-03-01 [PMID: 23417922] (ICC/IF, Mouse)

Parlet CP, Schlueter AJ. Mechanisms by Which Chronic Ethanol Feeding Impairs the Migratory Capacity of Cutaneous Dendritic Cells. Alcohol Clin Exp Res 2013-12-01 [PMID: 23895590] (FLOW, Mouse)

Choi SW, Hildebrandt GC, Olkiewicz KM et al. CCR1/CCL5 (RANTES) receptor-ligand interactions modulate allogeneic T-cell responses and graft-versus-host disease following stem-cell transplantation. Blood. 2007-11-01 [PMID: 17641205] (Flow-CS)

### Details:

Flow (Cell Surface): Fig 1C (splenocytes harvested from naive donor mice and transplant recipient mice). Splenic CD4+ and CD8+ T-cell expression of CCR1 was analyzed.

Vesosky B, Rottinghaus EK, Stromberg P et al. CCL5 participates in early protection against Mycobacterium tuberculosis. J Leukoc Biol. 2010-06-01 [PMID: 20371596] (Flow Cytometry Control, Flow - IC, Rabbit)

Demoule A, Divangahi M, Danialou G et al. Expression and regulation of CC class chemokines in the dystrophic (mdx) diaphragm. Am J Respir Cell Mol Biol. 2005-08-01 [PMID: 15860797] (IHC-Fr, Mouse)

## Procedures

### **Immunohistochemistry-Paraffin Protocol for CCR1 Antibody (NB100-56334)**

#### 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.



**Flow (Intracellular) Protocol for CCR1 Antibody (NB100-56334)**

## 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.
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. 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 10 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 10-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 amounts 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. 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 centrifuge 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 centrifuge 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.



**Immunocytochemistry/ Immunofluorescence Protocol for CCR1 Antibody (NB100-56334)**

## Immunocytochemistry Protocol

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

1. Remove culture medium and wash the cells briefly in PBS. Add 4% paraformaldehyde to the dish and fix at room temperature for 10 minutes.
2. Remove the paraformaldehyde and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 2 min.
4. Remove the permeabilization buffer and wash three times for 5 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 5 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 5 minutes each.
10. Counter stain DNA with DAPI if required.

**Western Blot Protocol for CCR1 Antibody (NB100-56334)**

## Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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 manufacturer's instructions.





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### **Products Related to NB100-56334**

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NBL1-08887	CCR1 Overexpression 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

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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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