Product Datasheet

CCR2 Antibody - BSA Free
NBP1-48337

Unit Size: 0.1 ml

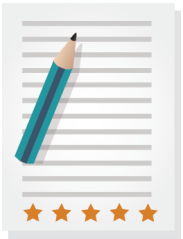
Store at -20C. Avoid freeze-thaw cycles.

www.novusbio.com technical@novusbio.com

Publications: 11

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NBP1-48337

Updated 10/23/2024 v.20.1



NBP1-48337

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

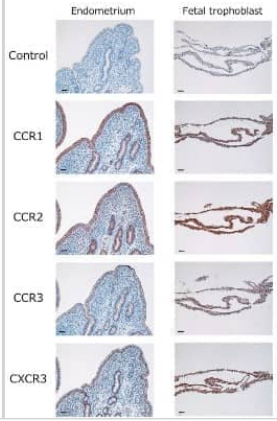
Host	Rabbit
Gene ID	729230
Gene Symbol	CCR2
Species	Human, Rat, Bovine, Feline
Reactivity Notes	Feline reactivity reported in scientific literature (PMID: 30395163). Use in Bovine reported in scientific literature (PMID:31761882). Reactivity with Rat reported by verified customer. The immunogen sequence similarity with other species: Rhesus monkey and several other primate species (100%), Mouse (69%).
Immunogen	Synthetic peptide made to an N-terminal portion of the human CCR2 protein (within residues 20-100). [Swiss-Prot# P41597]

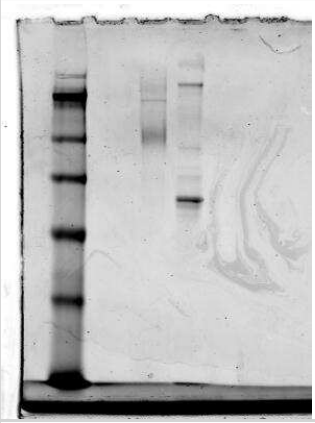
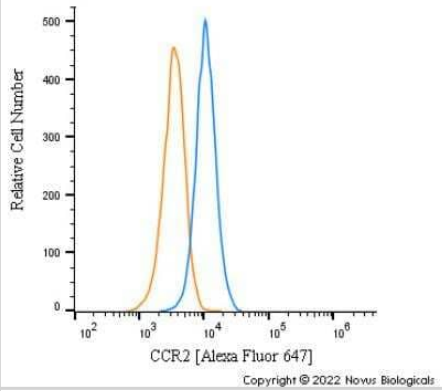
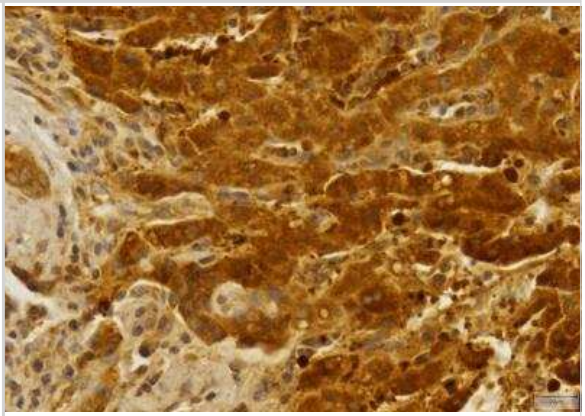
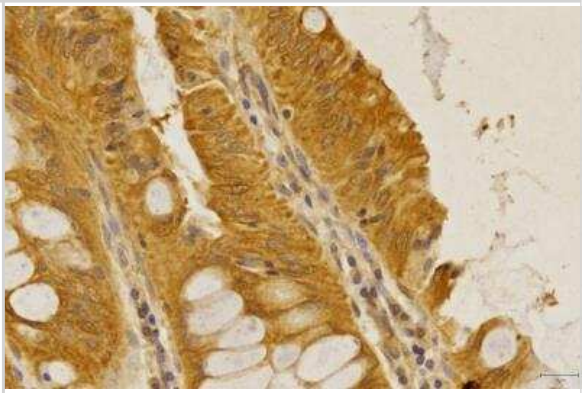
Product Application Details

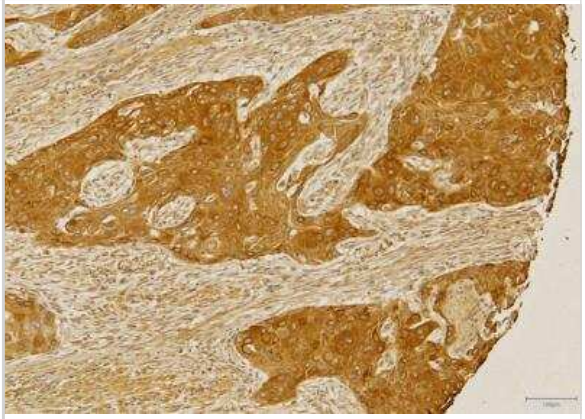
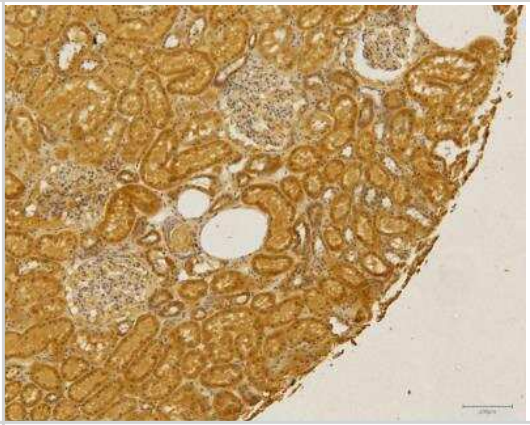
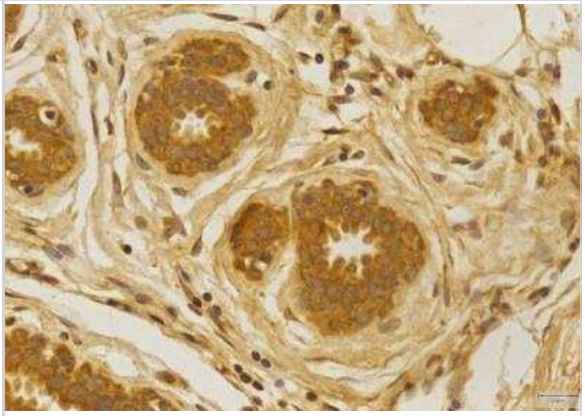
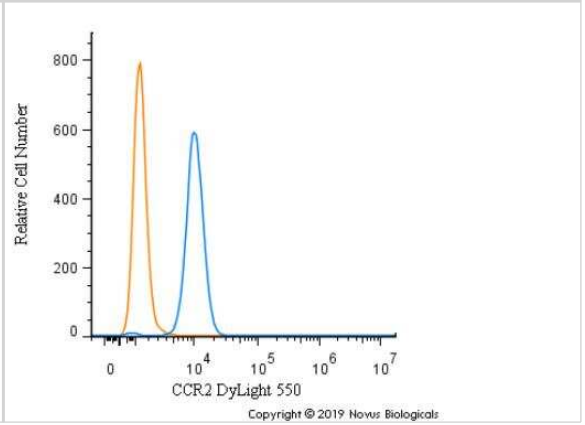
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 2 ug/ml, Flow Cytometry 5 ug/ml, Immunohistochemistry 5 ug/ml, Immunocytochemistry/ Immunofluorescence 1:50, Immunohistochemistry-Paraffin 5 ug/ml
Application Notes	This CCR2 antibody is useful for Flow Cytometry, Immunohistochemistry-Paraffin and Immunocytochemistry/Immunofluorescence applications. Some customers have also reported success using this antibody for Western Blot on rat and human lysates.

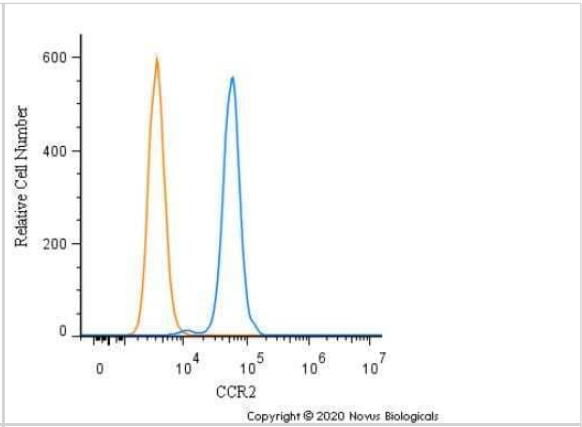
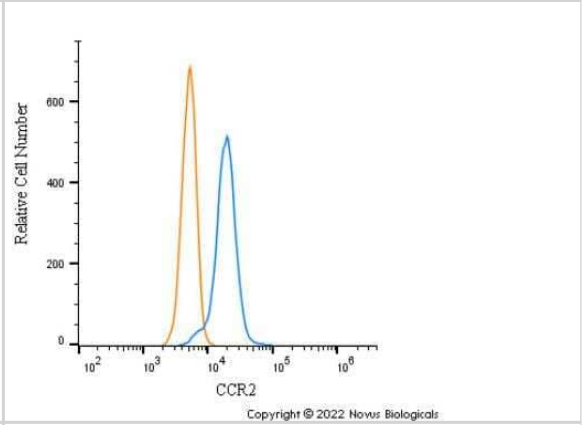
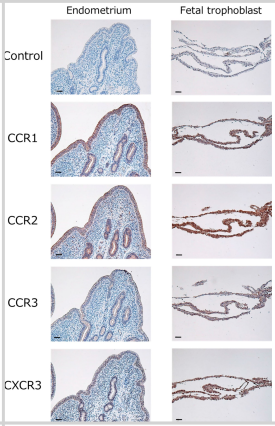
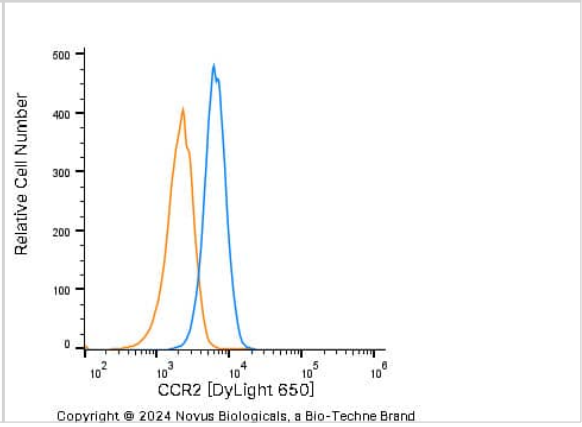
Images

Immunohistochemistry: CCR2 Antibody [NBP1-48337] - Localization of CCR1 (binds to CCL8, CCL14, and CCL16), CCR2 (binds to CCL2, CCL8, and CCL16), CCR3 (binds to CCL11), and CXCR3 (binds to CXCL10) in the bovine endometrium and fetal trophoblast obtained from cows in their 18th day of pregnancy. Intensive immunoreactivity was observed in endometrial epithelial cells, glandular epithelial cells, or fetal trophoblast. No positive immunoreactivity was observed in the negative control (Control). Scale bar = 50 um. Image collected and cropped by CiteAb from the following publication (https://www.mdpi.com/1422-0067/18/4/742), licensed under a CC-BY license.



Western Blot: CCR2 Antibody [NBP1-48337] - Analysis of CCR2 in rat lysate. Image courtesy of product review submitted by Rasha Elbaz.	
Flow Cytometry: CCR2 Antibody - BSA Free [NBP1-48337] - An intracellular stain was performed on U2-OS cells with CCR2 NBP1-48337AF647 (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 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Immunohistochemistry-Paraffin: CCR2 Antibody [NBP1-48337] - Analysis of FFPE tissue section of human liver cancer using 5 ug/ml concentration of CCR2 antibody. Intense cytoplasmic-membranous immune-staining was observed in the cancerous hepatocytes, whereas the staining was relatively weak in the cells adjacent to the fibrotic areas. [Magnification 40X]	
Immunohistochemistry-Paraffin: CCR2 Antibody [NBP1-48337] - Analysis of FFPE tissue section of human rectal adenocarcinoma using 5 ug/ml concentration of CCR2 antibody. The cancerous cells in the section developed a uniform but specific CCR2 immunopositivity whereas a weak staining was seen in the tumor stroma cells. [Magnification 40X]	

Immunohistochemistry-Paraffin: CCR2 Antibody [NBP1-48337] - Analysis of FFPE section of human esophageal squamous cell carcinoma (SCC) using 5 ug/ml concentration of CCR2 antibody. Strong cytoplasmic-membranous immune-staining was observed in SCC cells and a relatively weaker staining was seen in the tumor stroma cells. [Magnification 10X]	
Immunohistochemistry-Paraffin: CCR2 Antibody [NBP1-48337] - Analysis of FFPE tissue section of human normal kidney using 5 ug/ml concentration of CCR2 antibody. Strong cytoplasmic immunostaining was observed in the various renal cells (especially from tubules/ducts epithelium). The cells of Bowman's capsule depicted a very weak cytoplasmic staining for CCR2 protein. [Magnification 10X]	
Immunohistochemistry-Paraffin: CCR2 Antibody [NBP1-48337] - Analysis of FFPE tissue section of human normal breast using 5 ug/ml concentration of CCR2 antibody. The breast ductal/acinar epithelial cells showed a strong cytoplasmic-membranous CCR2 immune-positivity, whereas the intra-lobular connective tissue depicted weak staining. [Magnification 40X]	
Flow Cytometry: CCR2 Antibody [NBP1-48337] - An intracellular stain was performed on THP-1 cells with CCR Antibody NBP1-48337R (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 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 550.	

Flow Cytometry: CCR2 Antibody [NBP1-48337] - An intracellular stain was performed on THP-1 cells with CCR2 Antibody NBP1-48337 (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 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: CCR2 Antibody - BSA Free [NBP1-48337] - An intracellular stain was performed on U2-OS cells with CCR2 NBP1-48337 (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 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Localization of CCR1 (binds to CCL8, CCL14, and CCL16), CCR2 (binds to CCL2, CCL8, and CCL16), CCR3 (binds to CCL11), and CXCR3 (binds to CXCL10) in the bovine endometrium and fetal trophoblast obtained from cows in their 18th day of pregnancy. Intensive immunoreactivity was observed in endometrial epithelial cells, glandular epithelial cells, or fetal trophoblast. No positive immunoreactivity was observed in the negative control (Control). Scale bar = 50 µm.	
THP-1 human acute monocytic leukemia cell line was stained with Rabbit anti-CCR2 Affinity-purified Polyclonal Antibody conjugated to DyLight 650 (Catalog # NBP1-48337C, blue histogram) or matched control antibody (Catalog #NBP2-24891C, orange histogram).	

Publications

Li L, Liu Y, Hu W et al. Peripheral CCL2 induces inflammatory pain via regulation of Ih currents in small diameter DRG neurons Frontiers in molecular neuroscience 2023-10-04 [PMID: 37860084] (IHC, Mouse)

Soma K, Hitomi S, Hayashi Y et al. Neonatal Injury Modulates Incisional Pain Sensitivity in Adulthood: An Animal Study Neuroscience 2023-03-22 [PMID: 36958596] (IHC, Rat)

Tanaka T, Okuda H, Isonishi A et al. Dermal macrophages set pain sensitivity by modulating the amount of tissue NGF through an SNX25-Nrf2 pathway Nature immunology 2023-01-26 [PMID: 36703006] (IHC-Fr, Mouse)

Details:
Dilution used 1:500

Hummitzsch L, Zitta K, Fritze L Et al. Effects of remote ischemic preconditioning (RIPC) and chronic remote ischemic preconditioning (cRIPC) on levels of plasma cytokines, cell surface characteristics of monocytes and in-vitro angiogenesis: a pilot study Basic research in cardiology 2021-10-14 [PMID: 34651218] (ICC/IF, Human)

Mao Y, Lv X, Xu W Et al. Identification and validation of candidate genes dysregulated in alveolar macrophages of acute respiratory distress syndrome PeerJ 2021-10-26 [PMID: 34754619] (WB, Human)

Fang WB, Sofia Acevedo D, Smart C et al. Expression of CCL2/CCR2 signaling proteins in breast carcinoma cells is associated with invasive progression Sci Rep 2021-04-23 [PMID: 33888841] (IF/IHC, Human)

Details:
Citation using the PE format of this antibody.

KorimovA A, DubovY P N-Formylated Peptide Induces Increased Expression of Both Formyl Peptide Receptor 2 (Fpr2) and Toll-Like Receptor 9 (TLR9) in Schwannoma Cells-An In Vitro Model for Early Inflammatory Profiling of Schwann Cells Cells 2020-12-11 [PMID: 33322305] (WB, Rat)

Hirayama H, Sakumoto R, Koyama K et al. Expression of C-C motif chemokines and their receptors in bovine placentomes at spontaneous and induced parturition J. Reprod. Dev. 2020-02-14 [PMID: 31761882] (IHC-P, Bovine)

Kwon Ji Ye, Lee Seung Hoon, Na Hyun-Sik et al. Kartogenin inhibits pain behavior, chondrocyte inflammation, and attenuates osteoarthritis progression in mice through induction of IL-10. Scientific Reports 2018-09-14 [PMID: 30218055] (IF/IHC, Rat)

Rojo JL, Jaworski JP, Peluffo MC. Direct role of the C-C motif chemokine receptor 2/monocyte chemoattractant protein 1 system in the feline cumulus oocyte complex. Biol. Reprod. 2018-11-03 [PMID: 30395163] (ICC/IF, Feline)

Sakumoto R, Hayashi KG, Fujii S et al. Possible Roles of CC- and CXC-Chemokines in Regulating Bovine Endometrial Function during Early Pregnancy. Int J Mol Sci 2017-03-31 [PMID: 28362325] (IHC-P, Human)

Procedures

Western Blot protocol for CCR2 Antibody (NBP1-48337)

Western Blot Protocol
1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-CCR2 primary antibody (NBP1-48337) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG 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 [TBS + 0.1% Tween] 3 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 (Pierce ECL).
Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Flow (Intracellular) Protocol for CCR2 Antibody (NBP1-48337)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 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 uL 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 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL 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 permeablization 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 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabization buffer to every 1 x 106 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 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug 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 uL 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 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Immunohistochemistry-Paraffin Protocol for CCR2 Antibody (NBP1-48337)
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 all the time).

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.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NBP1-48337

NB800-PC1	HeLa Whole Cell Lysate
NBP1-48337PEP	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

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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NBP1-48337

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications