# **Product Datasheet**

# CCR5 Antibody - BSA Free NBP2-31374

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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#### NBP2-31374

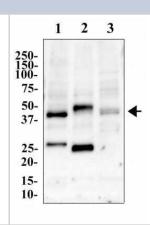
CCR5 Antibody - BSA Free

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	0.05% Sodium Azide
Isotype	IgG
Purity	Protein A purified
Buffer	PBS
Product Description	
Host	Rabbit
Gene ID	1234
Gene Symbol	CCR5
Species	Human, Mouse, Monkey
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 21305529). Rhesus macaque reactivity reported in a verified customer review.
Immunogen	Partial recombinant protein made to an N-terminal portion of the human CCR5 protein (between amino acids 1-350) [Uniprot: P51681]
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 2 ug/ml, Simple Western 1:50, Immunohistochemistry 5 ug/ml, Immunohistochemistry-Paraffin 5 ug/ml
Application Notes	In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.  See <u>Simple Western Antibody Database</u> for Simple Western validation: Tested in MCF-7 lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:50, apparent

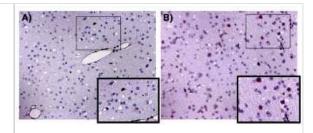
# **Images**

Western Blot: CCR5 Antibody [NBP2-31374] - Image of anti-CCR5 antibody. Whole cell protein from Daudi (1), K562 (2) and THP-1 (3) cell lines were separated by SDS-PAGE and protein transfered to PVDF. The membrane was probed with anti-CCR5 antibody at 2 ug/mL and detected with an anti-rabbit-HRP secondary antibody and chemiluminescence. CCR5 is shown at 40 kDa.

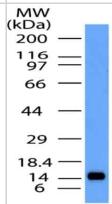
MW was 41 kDa.



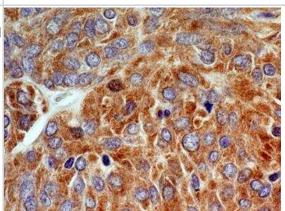
Immunohistochemistry-Paraffin: CCR5 Antibody [NBP2-31374] - Detection of CCR5 and virus on brain tissue. CCR5 expression was analyzed using Immunohistochemistry on the layer III of frontal cortex from macaques that were uninfected (A) and untreated controls, and Meth-only treated (B). Pictures were at 16x magnification. Sites of interest (rectangles) were further magnified. Image collected and cropped by CiteAb from the following publication (https://bmcimmunol.biomedcentral.com/articles/10.1186/s12865-016-0145-0), licensed under a CC-BY license.



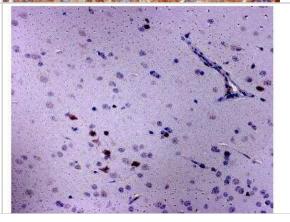
Western Blot: CCR5 Antibody [NBP2-31374] - Detection of CCR5 partial recombinant protein by using CCR5 antibody at a concentration of 0.5 ug/mL.



Immunohistochemistry-Paraffin: CCR5 Antibody [NBP2-31374] - Analysis of CCR5 protein in a section of malignant stromal tumor of small bowel from human using CCR5 antibody at a concentration of 5 ug/mL.



Immunohistochemistry-Paraffin: CCR5 Antibody [NBP2-31374] - Staining in a Rhesus macaque brain. IHC-P image submitted by a verified customer review.



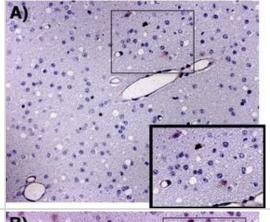
Immunohistochemistry-Paraffin: CCR5 Antibody [NBP2-31374] - Human skin section. IHC-P image submitted by a verified customer review.



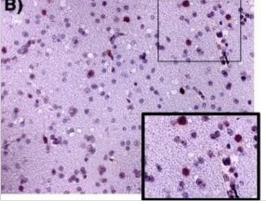
Simple Western: CCR5 Antibody [NBP2-31374] - Lane view shows a specific band for CCR5 in 0.5 mg/mL of MCF-7 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



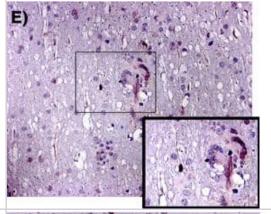
Immunohistochemistry: CCR5 Antibody [NBP2-31374] - Detection of CCR5 & virus on brain tissue. CCR5 expression was analyzed using Immunohistochemistry on the layer III of frontal cortex from macaques that were a Uninfected & untreated controls, b Meth-only treated, c SIV-only infected, & d SIV-infected & Meth-treated. e SIV-infected & Meth-treated. Pictures were at 16× magnification. Sites of interest (rectangles) were further magnified. f Brain viral load (Student's t test) Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27107567), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



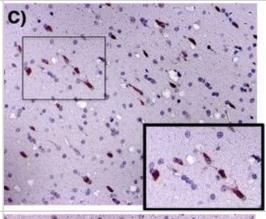
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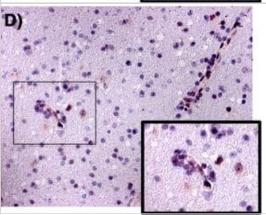
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#### **Publications**

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]

Liu B, Li A, Liu Y et al. Identification of Distinct Immune Signatures and Chemokine Networks in Scalp Inflammatory Diseases Research Square 2023-06-26 (IHC-P, Human)

Guma Monica, Ronacher Lisa M, Firestein Gary S et al. JNK-1 deficiency limits macrophage-mediated antigen-induced arthritis. Arthritis and Rheumatism 2011-01-01 [PMID: 21305529] (WB, Mouse)

Najera JA, Bustamante EA, Bortell N et al. Methamphetamine abuse affects gene expression in brain-derived microglia of SIV-infected macaques to enhance inflammation and promote virus targets. BMC Immunol 2016-04-23 [PMID: 27107567] (IF/IHC)



#### **Procedures**

#### Western Blot protocol for CCR5 Antibody (NBP2-31374)

CCR5 Antibody:

Western Blot Protocol

- 1. Perform SDS-PAGE on samples to be analyzed, loading 25 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 anti-CCR2 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 protocol for CCR5 Antibody (NBP2-31374)

CCR5 Antibody:

- 1. Deparaffinize the tissue sections by immersing the slides in Xylene with two changes for 10 min each. Sections should not get dried at any stage from this point.
- 2. Rehydrate the tissue sections by immersing the slides in decreasing grades of ethanol as follows:
- a. Immerse in 100% ethanol with 2 changes for 5 minutes each
- b. Immerse in 95% ethanol with 2 changes for 5 minutes each
- c. Immerse in 90% ethanol for 5 minutes
- d. Immerse in 70% ethanol for 5 minutes
- e. Immerse in 50% ethanol for 5 minutes
- f. Immerse in distilled water for 5 minutes
- 3. Antigen Retrieval (Microwave Method):
- a. Immerse the slides in a microwave compatible tray containing 10 mM Sodium Citrate buffer (pH 6.0) with 0.05% Tween 20.
- b. Boil the slides and maintain the sub-boiling temperature for 5 minutes in the microwave. Thereafter, take out the tray very carefully and cool it at room temperature (RT) for about 30 minutes.
- c. Wash the slides 3 times, 3 minutes each by immersing them in TBST (Tris Buffered Saline having 0.05% Tween 20).
- Quenching of Endogenous Peroxidase:
- a. Incubate the slides in 3% hydrogen peroxide prepared in methanol for 15 minutes (at RT, in dark conditions).
- b. Wash the slides in TBST 3 times, 3 minutes each.
- Protein Blocking:
- a. Incubate the sections with background sniper solution at RT for 15 minutes (Biocare Medicals, USA).
- b. Wash the sections 3 times, 3 min each by immersing the slides in TBST.
- 6. Primary Antibody:
- a. Dilute the primary antibody at 5ug/ml concentration using PBS as a diluent.
- b. Incubate the sections with diluted primary antibody for 90 minutes at RT in a humidified chamber.
- c. Thereafter, wash the slides 4 times, 5 minutes each with TBST.
- 7. Probe (Secondary Reagent):
- a. Incubate with MACH 1 Mouse probe for 15 minutes at RT.
- b. Incubate for 30 min at room temperature with HRP-Polymer (Biocare Medical, USA).
- c. Wash the slides with TBST 4 times, 5 minutes each
- 8. Chromogen:
- a. Mix 32ul of DAB Chromogen with 1 ml of DAB substrate buffer (Biocare Medical, USA).
- b. Apply 200ul DAB mixture/section and incubate at RT in dark conditions (few seconds 5 minutes).
- c. As soon as an appropriate color develops, rinse the slides with deionized water (2-3 brief rinses).
- 9. Counter stain:
- a. Counter stain with Hematoxylin for 30 seconds (Vector Labs, USA).
- b. Wash in deionized water for 1-2 minutes to clear the extra stain.
- c. Incubate the slides in bluing solution or Scott's water twice for 2 minutes each time.
- 10. Dehydrate the sections in increasing grades of alcohols:
- a. 50% alcohol for 1 minute
- b. 70% for 1 minute
- c. 90% for 1 minute
- d. 95% for 1 minute
- e. 100% for 1 minute
- f. Xylene with 2 changes for 2 minutes each
- 11. Mount with DPX mount and cover-slip glass (Fisher Scientific, USA), carefully not allowing any air bubbles to enter.

NOTE:- This protocol is provided as a reference tool only. Depending upon the type of tissues /tissue processing and reagents employed, the end user will need to optimize the final conditions for achieving an expected staining.





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# **Products Related to NBP2-31374**

NBP2-09009 CCR5 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

#### 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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