

# Product Datasheet

## CCL2/MCP1 Antibody (2D8) - BSA Free NBP2-22115

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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**NBP2-22115**

CCL2/MCP1 Antibody (2D8) - BSA Free

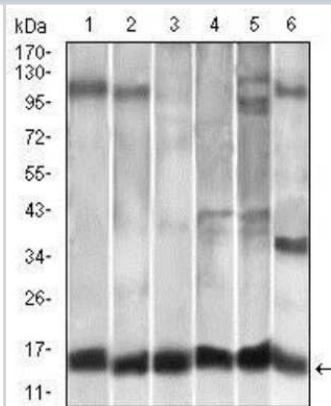
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	2D8
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein A or G purified
Buffer	PBS
Target Molecular Weight	11 kDa

Product Description	
Description	Novus Biologicals Mouse CCL2/MCP1 Antibody (2D8) - BSA Free (NBP2-22115) is a monoclonal antibody validated for use in IHC, WB, ELISA, Flow and ICC/IF. Anti-CCL2/MCP1 Antibody: Cited in 27 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	6347
Gene Symbol	CCL2
Species	Human, Mouse, Rat, Feline, Primate
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 29286133).
Immunogen	Full length human MCP1 expressed in E. coli. [Uniprot: P13500]

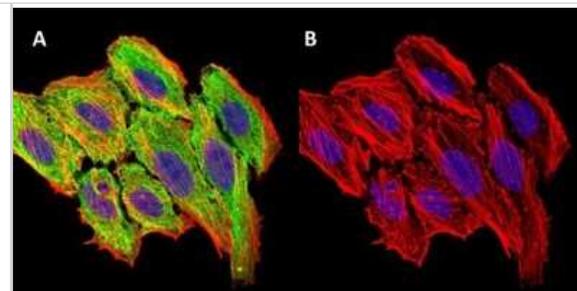
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1 - 3 ug/ml, Flow Cytometry 1:200-1:400, ELISA 1:10000, Immunohistochemistry 1:200-1:1000, Immunocytochemistry/ Immunofluorescence 1:200-1:1000, Immunohistochemistry-Paraffin 1:200-1:1000

**Images**

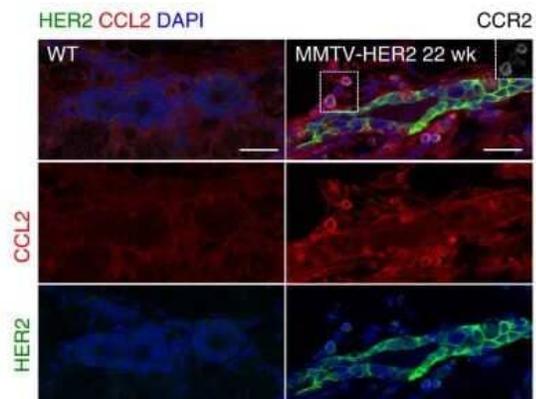
Western Blot: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - Analysis using MCP1 mouse mAb against A549 (1), HeLa (2), Raw264.7 (3), L1210 (4), C6 (5), and COS-7 (6) cell lysates.



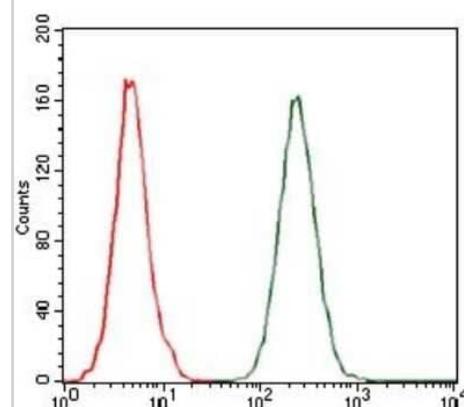
Immunocytochemistry/Immunofluorescence: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - Analysis of HepG2 cells using A) MCP1 mAb (green), DRAQ5 fluorescent DNA dye (blue), and Actin filaments have been labeled with Alexa Fluor-555 phalloidin (red). B) Staining without MCP1 mAb



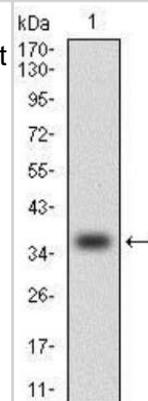
Immunohistochemistry: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - HER2 activates NF- $\kappa$ B and upregulates CCL2. MGs from FVB wild-type and 22-week-old MMTV-HER2 mice stained against CCL2, HER2, and CCR2. Bars: 25  $\mu$ m. Inset, CCR2 signal, zoom factor 1x; full image in Supplementary Fig. 5i. CCL2 intensity was quantified using ROI tool in Metamorph (Supplementary Fig. 5h). Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41467-017-02481-5>), licensed under a CC-BY license.



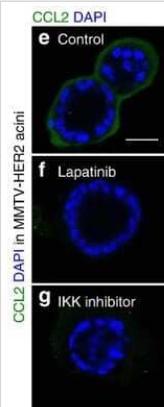
Flow Cytometry: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - Analysis of A549 cells using MCP1 mouse mAb (green) and negative control (red).



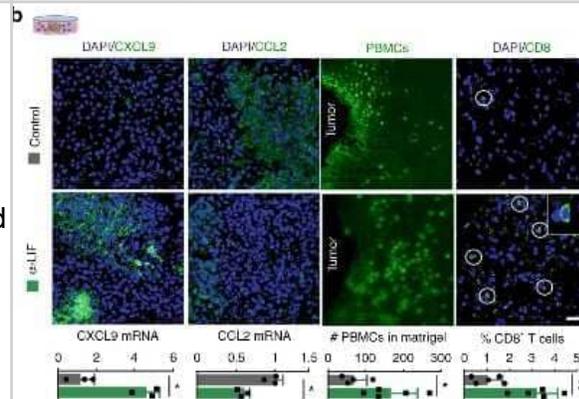
Western Blot: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - Analysis using MCP1 mAb against human MCP1 (AA: 1-99) recombinant protein. (Expected MW is 36.5 kDa)



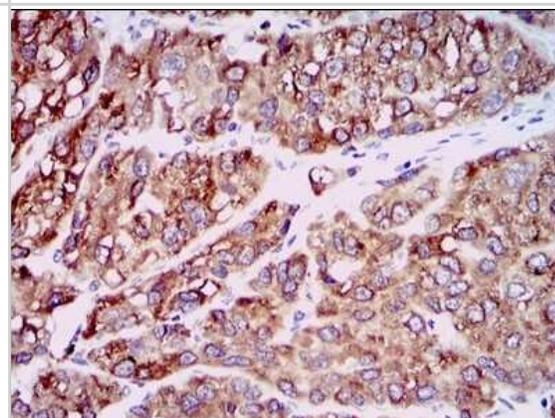
Immunocytochemistry/Immunofluorescence: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - CCL2 staining in acini cultures from MMTV-HER2 MGs (20-24 weeks) grown for 5 days and then DMSO-treated (vehicle control, e), 1uM lapatinib (f) or 1 uM IKKi compound A (g) for 24 hrs. Bar: 25 um. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41467-017-02481-5>), licensed under a CC-BY license.



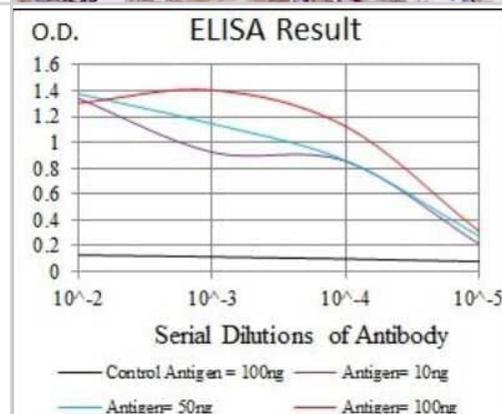
Immunohistochemistry: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - Organotypic specimens were treated with anti-LIF for 72 h and then cultured with PBMCs for 24 h. CXCL9 and CCL2 mRNA expression levels are shown. Representative images (patient 4, 5, 6) of CFSE-stained PBMCs into Matrigel containing GBM specimens and IF of the indicated factors in organotypic tissues are displayed. Bars represent quantification of five different fields of each condition. Data are presented as mean +/- SD. Scale bar, 20 um. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31186412/>) licensed under a CC-BY license.



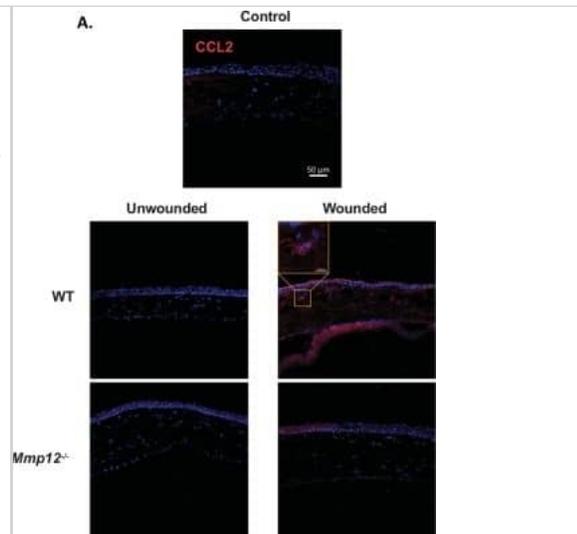
Immunohistochemistry-Paraffin: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - Analysis of paraffin-embedded liver cancer tissues using MCP1 mouse mAb with DAB staining.



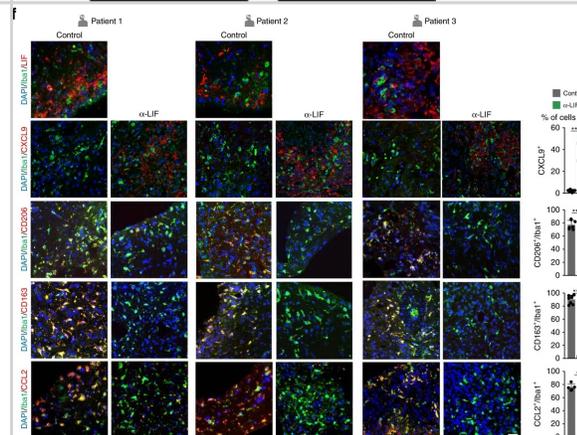
ELISA: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - Red: Control Antigen (100ng), Purple: Antigen (10ng), Green: Antigen (50ng), Blue: Antigen (100ng).



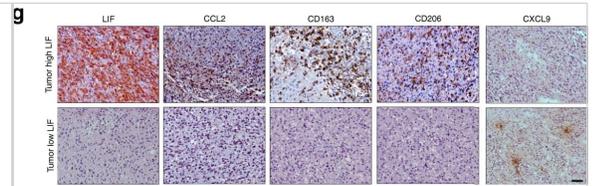
Immunocytochemistry/ Immunofluorescence: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - Expression patterns of CCL2 & CCR2 in unwounded & wounded corneas of WT & MMP12 KO mice. (A) Immunofluorescence of CCL2 chemokine & (B) its receptor CCR2 in unwounded & chemically wounded (2-days after injury) 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  $\mu$ m. 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.



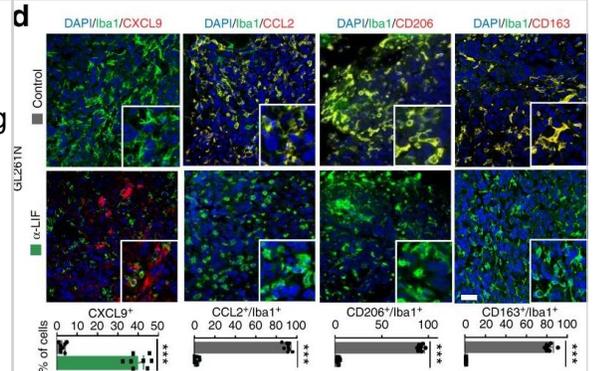
Immunocytochemistry/ Immunofluorescence: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - LIF represses CXCL9 through epigenetic silencing. a, b qRT-PCR analysis of the indicated genes in BMDMs. BMDMs were pre-incubated with 20 ng/ml LIF for 72 h & then stimulated with 5 ng/ml IFN $\gamma$  or 10  $\mu$ g/ml IL4 during 6 h (a) or with 0.1, 0.5, 1 & 5 ng/ml IFN $\gamma$  for 24 h (b). c CXCL9 ELISA from BMDMs pre-incubated with 20 ng/ml LIF & then stimulated with 0.1 ng/ml IFN $\gamma$  for 24 h. d CXCL9 ELISA from human CD11b<sup>+</sup>-sorted cells (77% CD11b<sup>+</sup> CD14<sup>+</sup>, see Supplementary Fig. 7b, c) from human GBM cultured with 20 ng/ml LIF for 72 h & then with 0.1 ng/ml IFN $\gamma$  for 24 h. e ChIP of Trimethyl-histone H3 (H3K27me3), EZH2 & acetyl-histone4 (H4ac) was performed in BMDMs treated with 20 ng/ml LIF for 72 h. Scheme shows the analysed CXCL9 promoter region. Representative data are presented as mean  $\pm$  SD. f Representative images of IF of the indicated markers in human GBM organotypic slices (patients 1, 2, 3) incubated with 10  $\mu$ g/ml anti-LIF for 3 days. Scale bar, 20  $\mu$ m. (see Supplementary Fig. 8). Bottom, percentage of double positive cells relative to Iba1<sup>+</sup> cells & percentage of CXCL9<sup>+</sup> cells relative to the total number of cells. Data are mean of all patients  $\pm$  SEM. Statistical analyses by Student's t-test or Mann-Whitney T-test. \*P < 0.05, \*\*P < 0.01; \*\*\*P < 0.001; \*\*\*\*P < 0.0001. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31186412>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



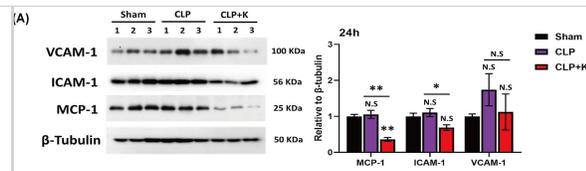
**Immunohistochemistry: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - LIF regulates CXCL9, CCL2, CD206, & CD163 in TAMs.** a Differential expression analysis of isolated CD11b+ cells from anti-LIF treated ID8 mice vs. control. Volcano plot representing the genes significantly (Q-value < 0.1) overexpressed (brown) & significantly underexpressed (turquoise). Heatmap representing the expression values of the indicated genes, each column represents a sample & each row a gene. The last column represents the log<sub>2</sub> fold change (log<sub>2</sub> FC) of gene expression. b mRNA expression for the indicated genes in isolated CD11b+ cells from anti-LIF treated or untreated ID8 & GL261N tumors. c Percentage & mean fluorescence intensity (MFI) of CCL2 & CXCL9 in TAMs (CD11b+ Ly6G<sup>-</sup> Ly6C<sup>-</sup>) from anti-LIF treated or untreated GL261N tumors. d Representative IF images of Iba1 & the indicated markers stainings of GL261N tumors (see Supplementary Fig. 4). Scale bar, 20 μm. Bottom, percentage of double positive cells relative to the TAM marker positive cells. CXCL9 quantification is relative to the total number of cells. e Tumor growth of GL261N in WT, CXCL9<sup>-/-</sup>, & CCL2<sup>-/-</sup> mice or mice treated with the indicated antibodies is shown as total flux (p/s). f Fold change (FC) of tumor infiltrating CD8<sup>+</sup> T cells in the indicated treatments. Data are mean ± SEM. g, h Representative IHC of the indicated markers from 20 GBM tumors. The degree of staining was quantified using H-score method. Correlations between LIF & CCL2, CD206, CD163, & CXCL9 with the R-squared coefficients (R<sup>2</sup>) were calculated (h). Statistical analyses by Mann-Whitney T-test. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; \*\*\*\*P < 0.0001. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31186412>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



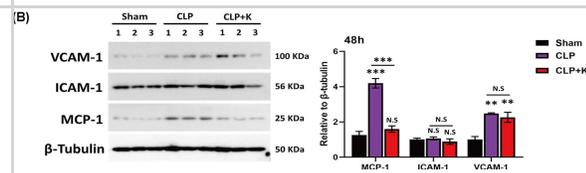
**Immunocytochemistry/ Immunofluorescence: CCL2/MCP1 Antibody (2D8) - BSA Free [NBP2-22115] - LIF regulates CXCL9, CCL2, CD206, & CD163 in TAMs.** a Differential expression analysis of isolated CD11b+ cells from anti-LIF treated ID8 mice vs. control. Volcano plot representing the genes significantly (Q-value < 0.1) overexpressed (brown) & significantly underexpressed (turquoise). Heatmap representing the expression values of the indicated genes, each column represents a sample & each row a gene. The last column represents the log<sub>2</sub> fold change (log<sub>2</sub> FC) of gene expression. b mRNA expression for the indicated genes in isolated CD11b+ cells from anti-LIF treated or untreated ID8 & GL261N tumors. c Percentage & mean fluorescence intensity (MFI) of CCL2 & CXCL9 in TAMs (CD11b+ Ly6G<sup>-</sup> Ly6C<sup>-</sup>) from anti-LIF treated or untreated GL261N tumors. d Representative IF images of Iba1 & the indicated markers stainings of GL261N tumors (see Supplementary Fig. 4). Scale bar, 20 μm. Bottom, percentage of double positive cells relative to the TAM marker positive cells. CXCL9 quantification is relative to the total number of cells. e Tumor growth of GL261N in WT, CXCL9<sup>-/-</sup>, & CCL2<sup>-/-</sup> mice or mice treated with the indicated antibodies is shown as total flux (p/s). f Fold change (FC) of tumor infiltrating CD8<sup>+</sup> T cells in the indicated treatments. Data are mean ± SEM. g, h Representative IHC of the indicated markers from 20 GBM tumors. The degree of staining was quantified using H-score method. Correlations between LIF & CCL2, CD206, CD163, & CXCL9 with the R-squared coefficients (R<sup>2</sup>) were calculated (h). Statistical analyses by Mann-Whitney T-test. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; \*\*\*\*P < 0.0001. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31186412>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



The production of MCP-1, VCAM-1, and ICAM-1 was significantly inhibited in Kaempferol treatment during sepsis(A) The protein expression level of MCP-1, VCAM-1, and ICAM-1 at 24 h; (B) protein expression level of MCP-1, VCAM-1, and ICAM-1 at 48 h in sham, saline-treated CLP mice, and kaempferol treated CLP mice (n = 3 per group, mean +/- S.E.M, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/37440431>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



The production of MCP-1, VCAM-1, and ICAM-1 was significantly inhibited in Kaempferol treatment during sepsis(A) The protein expression level of MCP-1, VCAM-1, and ICAM-1 at 24 h; (B) protein expression level of MCP-1, VCAM-1, and ICAM-1 at 48 h in sham, saline-treated CLP mice, and kaempferol treated CLP mice (n = 3 per group, mean +/- S.E.M, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/37440431>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Huang W, Hong S, Zhu X et al. Obesity Blunts the Effect of Mesenchymal Stem Cell-Derived Extracellular Vesicles  
Kidney International Reports 2023-06-21 [PMID: 37705914] (Human)

BL Phipps, U Suwannasua, J Lucero, NA Mitchell, AK Lund Vehicle emissions-exposure alters expression of systemic and tissue-specific components of the renin-angiotensin system and promotes outcomes associated with cardiovascular disease and obesity in wild-type C57BL/6 male mice Toxicology reports, 2021-04-15;8(0):846-862. 2021-04-15 [PMID: 33948438] (Human)

Xu Z, Wang X, Kuang W et al. Kaempferol improves acute kidney injury via inhibition of macrophage infiltration in septic mice Bioscience Reports 2023-07-26 [PMID: 37440431] (Human)

Dajnoki Z, Somogyi O, Medgyesi B Et al. Primary alterations during the development of hidradenitis suppurativa Journal of the European Academy of Dermatology and Venereology : JEADV 2021-11-01 [PMID: 34724272] (Human)

Y Jin, Y Liu, L Xu, J Xu, Y Xiong, Y Peng, K Ding, S Zheng, N Yang, Z Zhang, L Li, L Tan, H Song, J Fu Novel role for caspase 1 inhibitor VX765 in suppressing NLRP3 inflammasome assembly and atherosclerosis via promoting mitophagy and efferocytosis Cell Death & Disease, 2022-05-31;13(5):512. 2022-05-31 [PMID: 35641492] (Human)

Wang C, Cheng F, Han Z, Yan B et Al. Human-induced pluripotent stem cell-derived neural stem cell exosomes improve blood-brain barrier function after intracerebral hemorrhage by activating astrocytes via PI3K/AKT/MCP-1 axis Neural Regen Res 2024-05-31 [PMID: 38819064]

Quimbaya P, Garzon V, Bustos R et al. Real-time quantification of proteins secreted of conditioned media from mesenchymal stromal cells (MSC) in co-culture with hematopoietic progenitor cells Sensing and Bio-Sensing Research 2023-11-01 (SPR, Human)

Suzuki K, Ohe R, Kabasawa T et al. Histological spatial analysis on the induction of PD-L1+ macrophages by CD8+ T cells at the marginal microenvironment of triple-negative breast cancer Breast cancer (Tokyo, Japan) 2023-11-01 [PMID: 37792212] (IHC, Human)

Cui Y, Wang X, Xu Y et al. Ropivacaine promotes axon regeneration by regulating Nav1.8-mediated macrophage signaling after sciatic nerve injury in rats Anesthesiology 2023-09-05 [PMID: 37669448] (WB, Rat)

Aamir K, Sethi G, Afrin MR et al. Arjunolic acid modulate pancreatic dysfunction by ameliorating pattern recognition receptor and canonical Wnt pathway activation in type 2 diabetic rats Life sciences 2023-08-15 [PMID: 37307966]

Bae J, Lee JY, Shin E et al. The effects of the voglibose on non-alcoholic fatty liver disease in mice model Scientific reports 2022-08-10 [PMID: 35948569] (WB, Mouse)

Pasupulati A, Nishad R, Mukhi D et al. Growth hormone induces TNF-alpha in podocytes and contributes to monocyte-to-macrophage differentiation: Implications in Diabetic kidney disease Research Square 2022-02-02

More publications at <http://www.novusbio.com/NBP2-22115>

## Procedures

### Immunohistochemistry-Paraffin Protocol for CCL2/MCP1 Antibody (NBP2-22115)

#### 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.





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General: novus@novusbio.com

### **Products Related to NBP2-22115**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)

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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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