Product Datasheet

CCL2/MCP1 Antibody - BSA Free
NBP1-07035

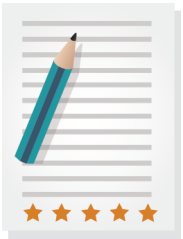
Unit Size: 0.1 ml

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

Reviews: 1 Publications: 26

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NBP1-07035

Updated 10/23/2024 v.20.1



NBP1-07035

CCL2/MCP1 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol
Target Molecular Weight	16 kDa

Product Description

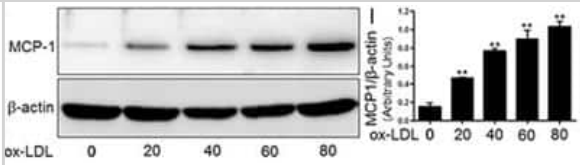
Host	Rabbit
Gene ID	6347
Gene Symbol	CCL2
Species	Human, Mouse, Rat
Immunogen	Synthetic peptide made to an internal portion of rat MCP1 (within residues 15-40). [Swiss-Prot# P14844]

Product Application Details

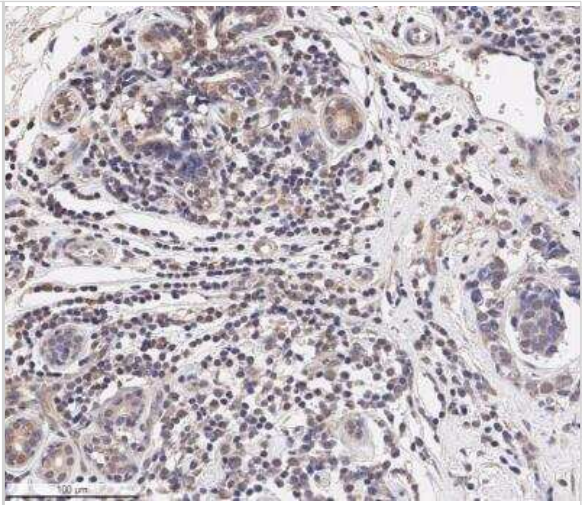
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Block/Neutralize
Recommended Dilutions	Western Blot 1:1000. Use reported in scientific literature (PMID 22402584), Immunohistochemistry 1:500-1:1000, Immunocytochemistry/ Immunofluorescence 1:2000, Immunohistochemistry-Paraffin 1:500-1:1000, Block/Neutralize reported in scientific literature (PMID 22778093)
Application Notes	In Western blot, a band is seen at ~16 kDa. In ICC/IF secretory vesicles staining was observed in HeLa cells.

Images

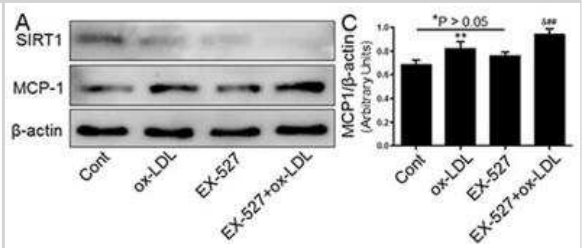
Western Blot: CCL2/MCP1 Antibody [NBP1-07035] - Western blot for MCP-1 protein was analyzed from the ox-LDL-stimulated THP-1 cells. B-actin was used as loading control (D-I). Scale bar: 20 um. Bar graph indicates the mean +/- SD (n = 3). *P < 0.05 and **P < 0.01 vs. Cont group (0 ug/mL of ox-LDL). Image collected and cropped by Citeab from the following publication (SIRT1 inhibition promotes atherosclerosis through impaired autophagy. Oncotarget (2017)) licensed under a CC-BY license.



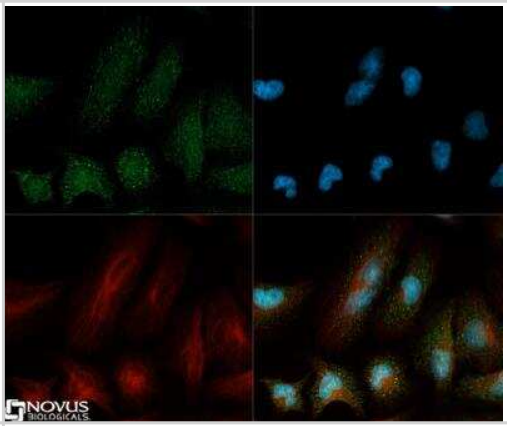
Immunohistochemistry-Paraffin: CCL2/MCP1 Antibody [NBP1-07035] - IHC analysis of a formalin-fixed paraffin-embedded (FFPE) human breast carcinoma tissue section using 1:1000 dilution of CCL2/MCP1 antibody (NBP1-07035) on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) with 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching using peroxide block. The sections were incubated with primary antibody for 30 minutes. Bond Polymer Refine Detection (Leica Biosystems) and DAB were used for signal detection which followed counterstaining with hematoxylin. Whole slide scanning and capturing of representative images (20X) were performed using Aperio AT2 (Leica Biosystems). This antibody generated a diffused cytoplasmic staining of CCL2 antigen in the cancer cells, stromal cells as well as the endothelial cells. The stroma itself showed a weak immunopositivity for CCL2. Staining was performed by Histowiz.



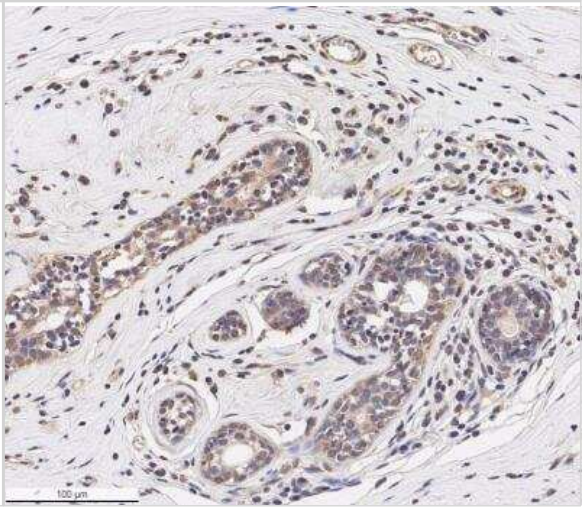
Western Blot: CCL2/MCP1 Antibody [NBP1-07035] - Inhibition of SIRT1 using EX-527 or SIRT1 siRNA transfection enhanced MCP-1 expression and foam cell formationHuman THP-1 macrophages were pretreated with EX-527 (2 uM, for 2 hrs) or SIRT1 siRNA (20 uM, for 24 hrs), and then exposed to 80 i1/4g/mL of ox-LDL for an additional 24 hrs. Western blot for SIRT1 and MCP-1 proteins were analyzed from the ox-LDL-stimulated THP-1 cells. I2-actin was used as loading control. Image collected and cropped by Citeab from the following publication (SIRT1 inhibition promotes atherosclerosis through impaired autophagy. Oncotarget (2017)) licensed under a CC-BY license.

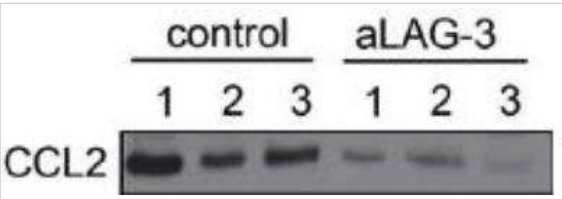
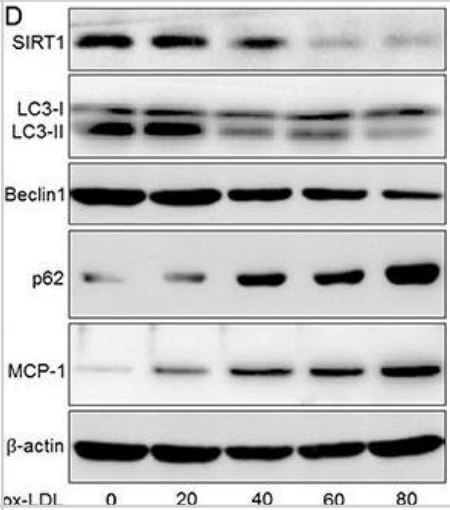
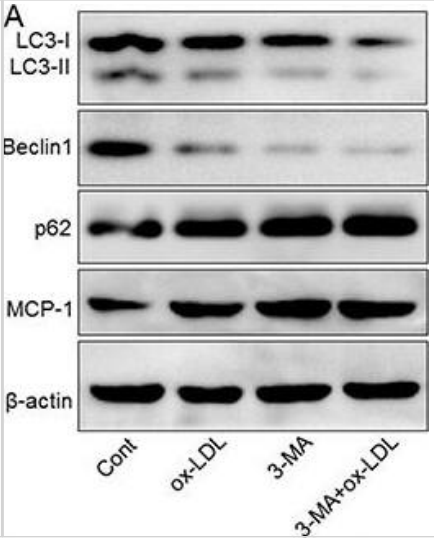


Immunocytochemistry/Immunofluorescence: CCL2/MCP1 Antibody [NBP1-07035] - MCP1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).

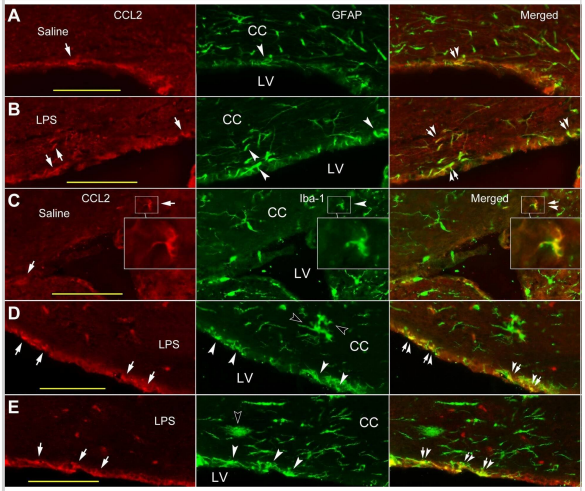


Immunohistochemistry-Paraffin: CCL2/MCP1 Antibody [NBP1-07035] - IHC analysis of a formalin-fixed paraffin-embedded (FFPE) human breast carcinoma tissue section using 1:1000 dilution of CCL2/MCP1 antibody (NBP1-07035) on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) with 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching using peroxide block. The sections were incubated with primary antibody for 30 minutes. Bond Polymer Refine Detection (Leica Biosystems) and DAB were used for signal detection which followed counterstaining with hematoxylin. Whole slide scanning and capturing of representative images (20X) were performed using Aperio AT2 (Leica Biosystems). This antibody generated a diffused cytoplasmic staining of CCL2 antigen in the cancer cells, stromal cells as well as the endothelial cells. The stroma itself showed a weak immunopositivity for CCL2. Staining was performed by Histowiz.

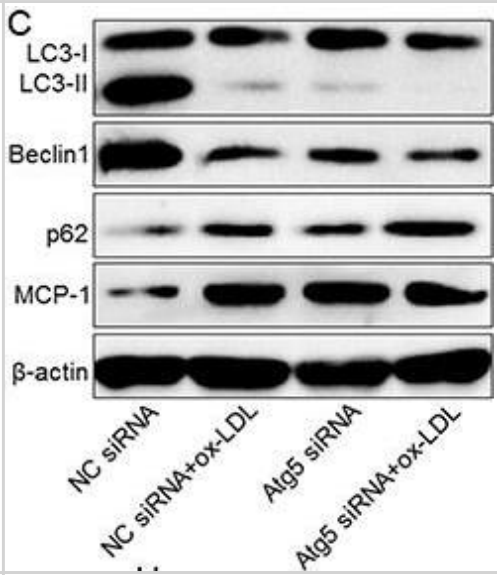


Western Blot: CCL2/MCP1 Antibody [NBP1-07035] - CCL2 expression level was evaluated in control group and aLAG-3 group.	
Western Blot: CCL2/MCP1 Antibody - BSA Free [NBP1-07035] - Ox-LDL induced macrophage foam cell formation, SIRT1 inhibition, autophagy impairment, & MCP-1 production in THP-1 cellsHuman THP-1 macrophages were exposed to 0, 20, 40, 60, & 80 µg/mL of ox-LDL for 24 hrs. Treated cells were photographed using light microscopy (A). The THP-1 macrophage-derived foam cell formation was determined using ORO staining method (B) & (C). Western blot for SIRT1, LC3, Beclin1, p62, & MCP-1 proteins were analyzed from the ox-LDL-stimulated THP-1 cells. β-actin was used as loading control (D-I). Scale bar: 20 µm. Bar graph indicates the mean ± SD (n = 3). *P < 0.05 & **P < 0.01 vs. Cont group (0 µg/mL of ox-LDL). Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.17691), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: CCL2/MCP1 Antibody - BSA Free [NBP1-07035] - Inhibition of autophagy with 3-MA led to increased foam cell formation & MCP-1 productionHuman THP-1 macrophages were pretreated with 3-MA (5 µM) for 2 hrs, & then exposed to 80 µg/mL of ox-LDL for an additional 24 hrs. Western blot for LC3, Beclin1, p62, & MCP-1 proteins were analyzed from the ox-LDL-stimulated THP-1 cells. β-actin was used as loading control (A-E). THP-1 macrophage-derived foam cell formation was determined using ORO staining method (F-G). Scale bar: 40 µm. Bar graph indicates the mean ± SD (n = 3). *P < 0.05 & **P < 0.01 vs. Cont group; #P < 0.05 & ##P < 0.01 vs. 3-MA group; &P < 0.05 & &&P < 0.01 represent significant differences between ox-LDL group & 3-MA+ox-LDL group. Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.17691), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

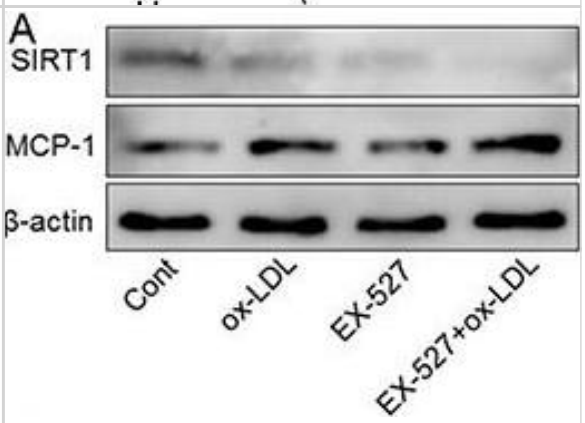
Immunocytochemistry/ Immunofluorescence: CCL2/MCP1 Antibody - BSA Free [NBP1-07035] - CCL2-ir cellular distribution in ependymal layers attached to the CC. A, B CCL2-ir (CCL2 panels, arrows) & GFAP (GFAP panels, arrowheads) double immunostained structures (Merged panels, arrow-arrowheads) are seen inlaid in the ependymal layers attached to the CC in both saline & LPS injected mice. C A CCL2-ir & Iba-1 double labeled soma (Merged, arrow-arrowhead) is seen at a corner of lateral ventricle, which seems be just next to an invaginated choroid plexus between the CC & basal ganglion (framed areas & insets). D, E Hyper-ramified & amoeba like Iba-1 labeled cells (Iba-1 panel, opened arrowheads) are observed in the CC of LPS injected mice, indicating their activated states. The CCL2-ir & Iba-1 double labeled structures are also regarded in the ependymal layers (Merged, arrow-arrowheads). Scare bar = 100 µm in all A– E Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35354428), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



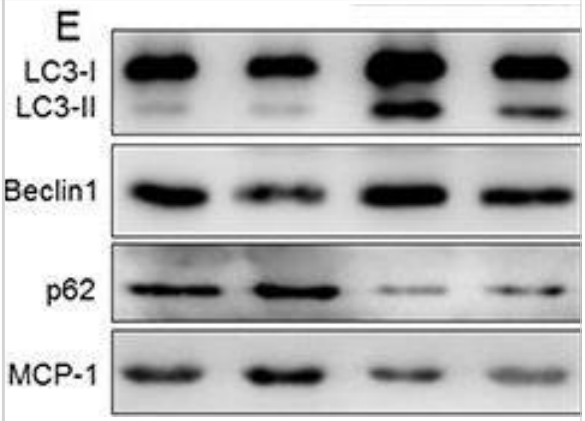
Western Blot: CCL2/MCP1 Antibody - BSA Free [NBP1-07035] - Inhibition of autophagy using Atg5 siRNA aggravated foam cell formation & MCP-1 expressionHuman THP-1 macrophages were pretreated with Atg5 siRNA (20 µM) for 24 hrs, & then exposed to 80 µg/mL of ox-LDL for an additional 24 hrs. Western blot forAtg5, LC3, Beclin1, p62, & MCP-1 proteins were analyzed from the ox-LDL-stimulated THP-1 cells. β-actin was used as loading control (A-F) & (I). THP-1 macrophage-derived foam cell formation was determined using ORO staining method (G) & (H). Scale bar: 40 µm. Bar graph indicates the mean ± SD (n = 3). *P < 0.05 & **P < 0.01 vs. NC siRNA group; #P < 0.05 & ##P < 0.01 vs. Atg5 siRNA group; &P < 0.05 & &&P < 0.01 represent significant differences between NC siRNA+ox-LDL group & Atg5 siRNA+ox-LDL group. Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.17691), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: CCL2/MCP1 Antibody - BSA Free [NBP1-07035] - Inhibition of SIRT1 using EX-527 or SIRT1 siRNA transfection enhanced MCP-1 expression & foam cell formationHuman THP-1 macrophages were pretreated with EX-527 (2 µM, for 2 hrs) or SIRT1 siRNA (20 µM, for 24 hrs), & then exposed to 80 µg/mL of ox-LDL for an additional 24 hrs. Western blot for SIRT1 & MCP-1 proteins were analyzed from the ox-LDL-stimulated THP-1 cells. β-actin was used as loading control (A-C) & (F-H). THP-1 macrophage-derived foam cell formation was determined using ORO staining method (D-E) & (I-J). Scale bar: 40 µm. Bar graph indicates the mean ± SD (n = 3). *P < 0.05 & **P < 0.01 vs. Cont group (NC siRNA group); #P < 0.05 & ##P < 0.01 vs. EX-527 group (SIRT1 siRNA group); &P < 0.05 & &&P < 0.01 represent significant differences between ox-LDL group (NC siRNA+ox-LDL group) & EX-527+ox-LDL group (SIRT1 siRNA+ox-LDL group). Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.17691), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: CCL2/MCP1 Antibody - BSA Free [NBP1-07035] - Overexpression of SIRT1 using adenoviral transfection reversed ox-LDL-induced macrophage foam cell formation & autophagy impairment in THP-1 cellsHuman THP-1 macrophages were transfected by SIRT1 over-expressing adenovirus (HBAD-SIRT1) or NC adenovirus (HBAD-GFP) for 24 hrs & then exposed to 80 μg/mL of ox-LDL for an additional 24 hrs. The transfected THP-1 cells were observed using an inverted fluorescence microscope (A) & then were harvested for transfection efficiency analysis by Western blot method (B). THP-1 macrophage-derived foam cell formation was determined using ORO staining method (C) & (D). Western blot for LC3, Beclin1, p62, & Atg5 proteins & immunoprecipitation for acetyl-Lys Atg5 were analyzed from the ox-LDL-stimulated THP-1 cells. β-actin was used as loading control (E-L). Scale bar: 40 μm. Bar graph indicates the mean ± SD (n = 3). *P < 0.05 & **P < 0.01 vs. HBAD-GFP group; #P < 0.05 & ##P < 0.01 vs. HBAD-SIRT1 group; &P < 0.05 & &&P < 0.01 represent significant differences between HBAD-GFP+ox-LDL group & HBAD-SIRT1+ox-LDL group. Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.17691), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Tanaka H, Kondo K, Fujita K Et al. HMGB1 signaling phosphorylates Ku70 and impairs DNA damage repair in Alzheimer's disease pathology Communications biology 2021-10-11 [PMID: 34635772]

Zhou Jiang, Qianpei He, Jackson Wezeman, Martin Darvas, Warren Ladiges A cocktail of rapamycin, acarbose, and phenylbutyrate prevents age-related cognitive decline in mice by targeting multiple aging pathways. GeroScience 2024-05-16 [PMID: 38755466]

Zhao H, Liu Z, Chen H et al. Identifying specific functional roles for senescence across cell types. Cell 2024-10-01 [PMID: 39368477]

Yitong Zhao, Masaki Goto, Nosratola D Vaziri, Mahyar Khazaeli, Han Liu, Nazli Farahanchi, Elham Khanifar, Ted Farzaneh, Patrick A Haslett, Hamid Moradi, Mangala M Soundarapandian RNA Interference Targeting Liver Angiopoietin-Like Protein 3 Protects from Nephrotic Syndrome in a Rat Model Via Amelioration of Pathologic Hypertriglyceridemia. The Journal of pharmacology and experimental therapeutics 2021-04-27 [PMID: 33443084]

T. Ollewagen, R.M. Benecke, C. Smith High species homology potentiates quantitative inflammation profiling in zebrafish using immunofluorescence Heliyon 2023-12-13 [PMID: 38187273]

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

Details:
HMC Stimulation

Wilkinson A, Hulme S, Kennedy J et al. The senescent secretome drives PLVAP expression in cultured human hepatic endothelial cells to promote monocyte transmigration iScience 2023-09-01 [PMID: 37810232]

Zhu X, Xie W, Zhang J et al. Sympathectomy decreases pain behaviors and nerve regeneration by downregulating monocyte chemokine CCL2 in dorsal root ganglia in the rat tibial nerve crush model Pain 2022-01-01 [PMID: 33941753] (Immunohistochemistry)

Merckx C, Zsch□ntzsch J, Meyer S et al. Exploring the Therapeutic Potential of Ectoine in Duchenne Muscular Dystrophy: Comparison with Taurine, a Supplement with Known Beneficial Effects in the mdx Mouse International Journal of Molecular Sciences 2022-08-24 [PMID: 36076964] (Immunocytochemistry/ Immunofluorescence)

Torres-Arévalo, Á;Nahuelpán, Y;Muñoz, K;Jara, C;Cappelli, C;Taracha-Wiśniewska, A;Quezada-Monrás, C;Martín, RS; A2BAR Antagonism Decreases the Glomerular Expression and Secretion of Chemoattractants for Monocytes and the Pro-Fibrotic M2 Macrophages Polarization during Diabetic Nephropathy International journal of molecular sciences 2023-06-29 [PMID: 37446007] (ELISA)

Lee C, Cho S, Jeong D Inhibition of miR-25 Ameliorates Cardiac Dysfunction and Fibrosis by Restoring KLF4 Expression Preprint 2023-07-11 [PMID: 37569807] (WB)

Andre AB, Rees KP, O'Connor S et al. Single cell analysis reveals satellite cell heterogeneity for proinflammatory chemokine expression Frontiers in cell and developmental biology 2023-03-27 [PMID: 37051469] (IHC-P, Mouse)

Details:
1:500 IHC-P dilution

More publications at http://www.novusbio.com/NBP1-07035

Procedures

Western Blot Protocol for MCP1 Antibody (NBP1-07035)

Procedure Guide for NBP1-07035 - MCP-1 Antibody
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-MCP-1 primary antibody (NBP1-07035) 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.

Immunocytochemistry/Immunofluorescence Protocol for MCP1 Antibody (NBP1-07035)

Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NBP1-07035

NBL1-08849	CCL2/MCP1 Overexpression Lysate
NBP1-07035PEP	CCL2/MCP1 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.

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