abcam

ab214819

Anti-MCP1 antibody [EPR21025]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR21025
Purification technique	Affinity purification Protein A
Specificity	Stimulation may be required for the detection of MCP1, as it is not constitutively expressed.
Concentration	0.489 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	1/50

Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/2000 - 1/10000
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Target data

See full target information CCL2

Function	Acts as a ligand for C-C chemokine receptor CCR2 (PubMed:10529171, PubMed:10587439, PubMed:9837883). Signals through binding and activation of CCR2 and induces a strong chemotactic response and mobilization of intracellular calcium ions (PubMed:10587439, PubMed:9837883). Exhibits a chemotactic activity for monocytes and basophils but not neutrophils or eosinophils (PubMed:8195247, PubMed:8627182, PubMed:9792674). May be involved in the recruitment of monocytes into the arterial wall during the disease process of atherosclerosis (PubMed:8107690).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	MCP-1 also known as CCL2 is a chemokine involved in the recruitment of monocytes to sites of injury and inflammation. This protein consists of 76 amino

	acids and has a molecular weight of about 11 kDa. MCP-1 is expressed in a wide range of cell types including endothelial cells fibroblasts and monocytes. It acts by binding to the chemokine receptor CCR2 stimulating intracellular signaling pathways that mediate cell migration.
Biological function summary	The role of MCP-1 is significant in guiding immune cells to areas requiring immune response. It does not form part of a complex but operates as a singular entity to affect immune signaling. MCP-1 influences the behavior of monocytes and macrophages by inducing chemotaxis and activating their effector functions. Through this mechanism it aids in the inflammatory response and tissue repair.
Pathways	MCP-1 participates in the inflammatory pathways by cooperating with key proteins like CCR2. It facilitates the JAK-STAT signaling pathway which is vital for transmitting extracellular information into cellular responses. The interaction of MCP-1 with CCR2 influences the activation of downstream proteins like STAT3 enabling various immune responses. MCP-1 thereby plays a central role in modulating inflammation-related pathways.
Associated diseases and disorders	MCP-1 has a connection to inflammatory bowel disease and atherosclerosis. In such conditions elevated levels of MCP-1 can exacerbate inflammatory responses by recruiting monocytes to dysfunctional sites worsening tissue damage. Through its interaction with CCR2 MCP-1 becomes a pivotal factor in chronic inflammation. Targeting this MCP-1/CCR2 axis can provide therapeutic opportunities for modulating disease progression in these inflammatory diseases.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

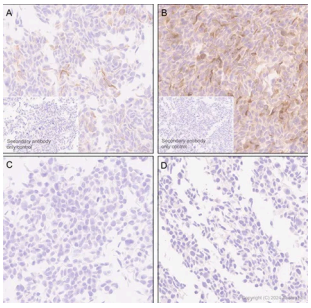
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:

11 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] (ab214819)

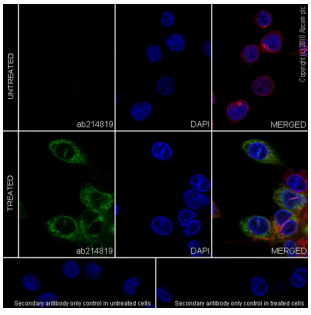
Immunohistochemical analysis of paraffin-embedded wild-type HeLa cell pellets and CCL2 knockout HeLa cell pellets labelling MCP1 with ab214819 at a 1/10000 dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) secondary antibody. Counter stained with Hematoxylin.

Image A: Wild-type Hela cell pellets treated with Brefeldin A (1µg/ml) for 3 hours.

Image B: Wild-type Hela cell pellets treated with TNF-alpha (TNFα, 20ng/ml) for 3 hours, 1µg/ml Brefeldin A was added for additional 3 hours.

Image C: CCL2 knockout HeLa cell pellets treated with Brefeldin A (1µg/ml) for 3 hours.

Image D: CCL2 knockout HeLa cell pellets treated with TNF-alpha (TNFα, 20ng/ml) for 3 hours, 1µg/ml Brefeldin A was added for additional 3 hours.
Positive staining on wild-type HeLa cell pellets treated with Brefeldin A (1µg/ml) for 3 hours (Image A) and wild-type HeLa cell pellets treated with TNF-alpha (TNFα, 20ng/ml) for 3 hours, 1µg/ml Brefeldin A was added for additional 3 hours (Image B); No staining on CCL2 knockout HeLa cell pellets treated with Brefeldin A (1µg/ml) for 3 hours (Image C) and CCL2 knockout HeLa cell pellets treated with TNF-alpha (TNFα, 20ng/ml) for 3 hours, 1µg/ml Brefeldin A was added for additional 3 hours (Image D). The section was incubated with ab214819 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

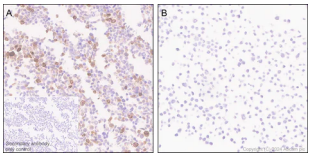


Immunocytochemistry/ Immunofluorescence - Anti-MCP1 antibody [EPR21025] (ab214819)

MCP1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MCP1 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized THP-1 (human monocytic leukemia cell line) cells, untreated or treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24 hours, then treated with 100ng/ml lipopolysaccharide (LPS) for 7 hours, with 1 µg/ml Brefeldin A (BFA) added after 4 hours, labeling MCP1 with ab214819 at 1/50 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in THP-1 treated cells.
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

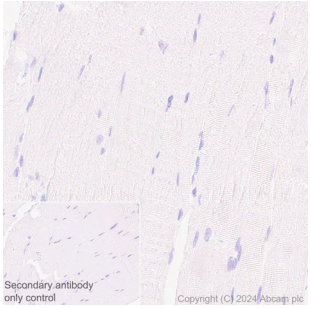


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] (ab214819)

Immunohistochemical analysis of paraffin-embedded THP-1 (human monocytic leukemia monocyte) cell pellets labelling MCP1 with ab214819 at a 1/10000 dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) secondary antibody. Counter stained with Hematoxylin.

Image A: THP-1 (human monocytic leukemia monocyte) cell pellets treated with 80nM Phorbol-12-myristate-13-acetate (PMA) for 24 hours, then added 100ng/ml Lipopolysaccharides (LPS) for 7 hours, 1µg/ml Brefeldin A was added for additional 3 hours.

Image B: Untreated THP-1 cell pellets.
Cytoplasmic staining on THP-1 cell pellets treated with 80nM Phorbol-12-myristate-13-acetate (PMA) for 24 hours, then added 100ng/ml Lipopolysaccharides (LPS) for 7 hours, 1µg/ml Brefeldin A was added for additional 3 hours (Image A); No staining on untreated THP-1 cell pellets (Image B). The section was incubated with ab214819 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

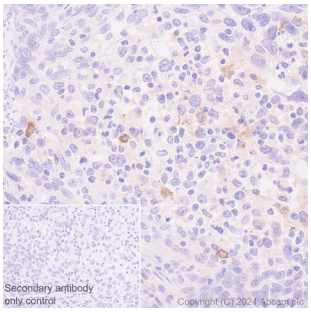


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] (ab214819)

Immunohistochemical analysis of paraffin-embedded human skeletal muscle sections labelling MCP1 with ab214819 at a 1/10000 dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) secondary antibody. Counter stained with Hematoxylin.

Negative control: no staining on human skeletal muscle.

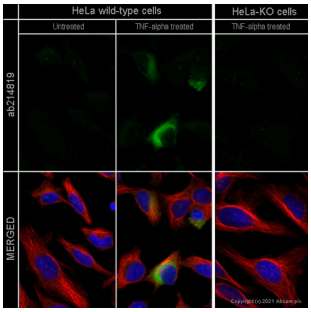
The section was incubated with ab214819 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] (ab214819)

Immunohistochemical analysis of paraffin-embedded human lung adenocarcinoma sections labelling MCP1 with ab214819 at a 1/2000 dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) secondary antibody. Counter stained with Hematoxylin.

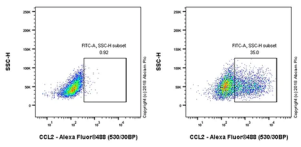
Positive staining on human lung adenocarcinoma. The section was incubated with ab214819 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunocytochemistry - Anti-MCP1 antibody [EPR21025] (ab214819)

MCP1 Immunocytochemistry staining using rabbit Anti-MCP1 antibody

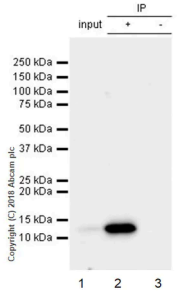
ab214819 staining MCP1 in wild-type and MCP1 knockout HeLa cells (ab255372), untreated or treated with TNF-alpha (20ng/ml, 6 hours) and Brefeldin A (1µg/mL, 3 hours). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab214819 at 1µg/ml concentration and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Flow Cytometry - Anti-MCP1 antibody [EPR21025] (ab214819)

MCP1 Flow Cytometry staining using rabbit Anti-MCP1 antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 0.1% Tween-20-permeabilized THP-1 (human monocytic leukemia cell line) cell line, treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h (Right) / Untreated control (Left)labeling MCP1 with ab214891 at 1/500 dilution. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-MCP1 antibody [EPR21025] (ab214819)

MCP1 was immunoprecipitated from 0.35 mg of THP-1 (human monocytic leukemia cell line) treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate with ab214819 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab214819 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1,000 dilution
Lane 1: THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate 10 µg (Input).

Lane 2: ab214819 IP in THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab214819 in THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate.

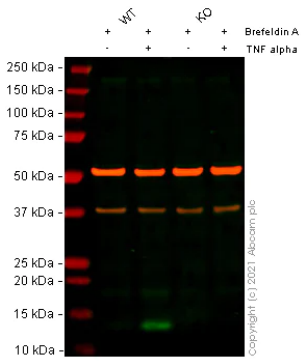
Blocking and dilution buffer: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-MCP1 antibody [EPR21025] (ab214819)

Predicted band size: 11 kDa, 14 kDa

Observed band size: 12 kDa

Exposure time: 30s



Western blot - Anti-MCP1 antibody [EPR21025] (ab214819)

MCP1 Western blot staining using rabbit Anti-MCP1 antibody

False colour image of Western blot: Anti-MCP1 antibody [EPR21025] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab214819 was shown to bind specifically to MCP1. A band was observed at 11 kDa in wild-type HeLa cell lysates with no signal observed at this size in ccl2 knockout cell line ab255372 (knockout cell lysate ab263807). To generate this image, wild-type and ccl2 knockout cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-MCP1 antibody [EPR21025] (ab214819) at 1/1000 dilution

Lane 1:
Wild-type HeLa Vehicle Control TNF alpha (0ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate at 20 µg

Lane 2:
Wild-type HeLa Treated TNF alpha (20ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate at 20 µg

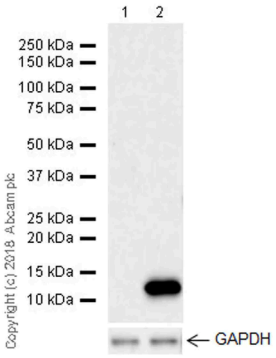
Lane 3:
CCL2 knockout HeLa Vehicle Control TNF alpha (0ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate at 20 µg

Lane 4:
CCL2 knockout HeLa Treated TNF alpha (20ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 11 kDa

Observed band size: 11 kDa



Western blot - Anti-MCP1 antibody [EPR21025] (ab214819)

MCP1 Western blot staining using rabbit Anti-MCP1 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-MCP1 antibody [EPR21025] (ab214819) at 1/1000 dilution

Lane 1:
Untreated THP-1 (human monocytic leukemia cell line) whole cell lysate at 20 µg

Lane 2:
THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24 hours, then treated with 100ng/ml lipopolysaccharide (LPS) for 7 hours, then with 1 µg/ml Brefeldin A (BFA) added after 4 hours, whole cell lysate at 20 µg

Secondary

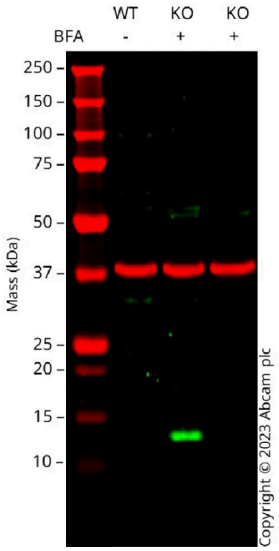
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 11 kDa

Observed band size: 11 kDa

Exposure time: 3min

Western blot - Anti-MCP1 antibody [EPR21025] (ab214819)



MCP1 Western blot staining using rabbit Anti-MCP1 antibody

Anti-CCL2 antibody [EPR21025] (ab214819) staining at 1/400 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab214819 was shown to bind specifically to CCL2. A band was observed at 13 kDa in wild-type A549 cell lysates with no signal observed at this size in CCL2 knockout cell line ab270478 (knockout cell lysate ab270501). To generate this image, wild-type and CCL2 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween$®$ 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution and Goat anti-Mouse IgG H&L 680RD at 1/80000 dilution
All lanes:
Western blot - Anti-MCP1 antibody [EPR21025] (ab214819)

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
Wild-type A549 Treated BFA (1 ug/mL, 3 h) cell lysate, at 20 µg

Lane 3:
CCL2 knockout A549 Treated BFA (1 ug/mL, 3 h) cell lysate at 20 µg

Observed band size: 13 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.