abcam

Product datasheet

Anti-MCP1 antibody [EPR21025] ab214819

敲除验证 重组 RabMAb

6 References 12 图像

概述

产品名称	Anti-MCP1抗体[EPR21025]
描述	兔单克隆抗体[EPR21025] to MCP1
宿主	Rabbit
特异性	Stimulation may be required for the detection of MCP1, as it is not constitutively expressed.
经测试应用	**适用于:** ICC/IF, Flow Cyt (Intra), IHC-P, WB, IP
种属反应性	**与反应:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: THP-1 treated with PMA (**ab120297**), LPS, and BFA whole cell lysate. ICC/IF: THP-1 cells treated with PMA (**ab120297**), LPS, and BFA, wild-type and MCP1 knockout HeLa cells (**ab255372**) treated with TNF-alpha (20ng/mL, 6 hours). Flow Cyt (intra): THP-1 treated with PMA (**ab120297**), LPS, and BFA. IP: THP-1 treated with PMA (**ab120297**), LPS, and BFA whole cell lysate. IHC: Human lung adenocarcinoma; THP-1 cell pellets treated with PMA, LPS, and BFA; Wild type HeLa cell pellets treated with BFA, and TNF-alpha.
常规说明	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯度	Protein A purified
克隆	单克隆
克隆编号	EPR21025
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab214819于以下的经测试应用**

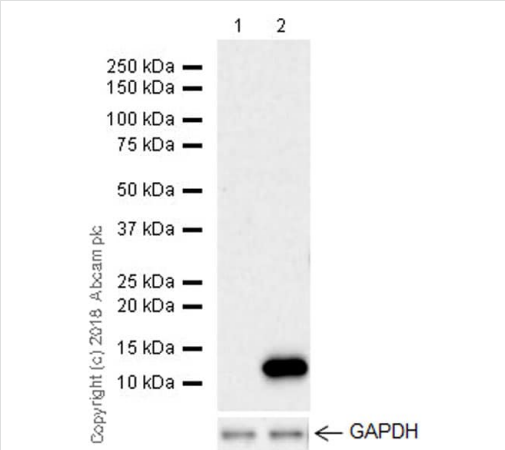
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		1/50.
Flow Cyt (Intra)		1/500.
IHC-P		1/2000 - 1/10000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 11 kDa (predicted molecular weight: 11 kDa).
IP		1/30.

靶标

功能	Chemotactic factor that attracts monocytes and basophils but not neutrophils or eosinophils. Augments monocyte anti-tumor activity. Has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates, like psoriasis, rheumatoid arthritis or atherosclerosis. May be involved in the recruitment of monocytes into the arterial wall during the disease process of atherosclerosis.
序列相似性	Belongs to the intercrine beta (chemokine CC) family.
翻译后修饰	Processing at the N-terminus can regulate receptor and target cell selectivity. Deletion of the N-terminal residue converts it from an activator of basophil to an eosinophil chemoattractant.
细胞定位	Secreted.

图片



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

All lanes : Anti-MCP1 antibody [EPR21025] (ab214819) at 1/1000 dilution

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

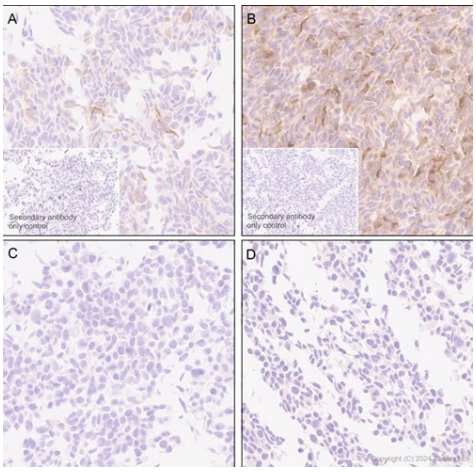
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 11 kDa
Observed band size: 11 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

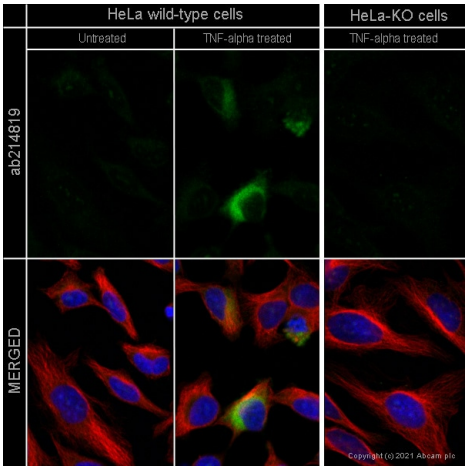


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] (ab214819)

Immunohistochemical analysis of paraffin-embedded wild-type 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.

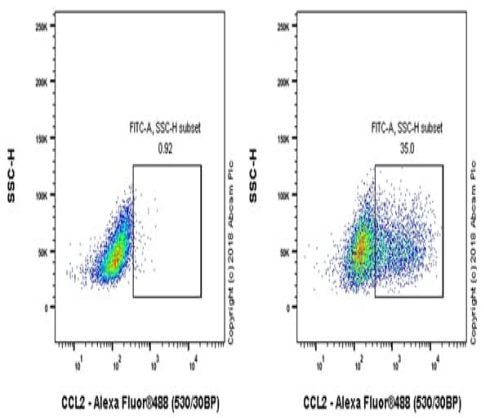
Image A: Wild-type Hela cell pellets treated with Brefeldin A (1µg/ml) for 3 hours.
Image B: Wild-type Hela cell pellets treated with TNF-alpha (TNFα, 20ng/ml) for 3 hours, 1µg/ml Brefeldin A was added for additional 3 hours.
Image C: CCL2 knockout HeLa cell pellets treated with Brefeldin A (1µg/ml) 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.



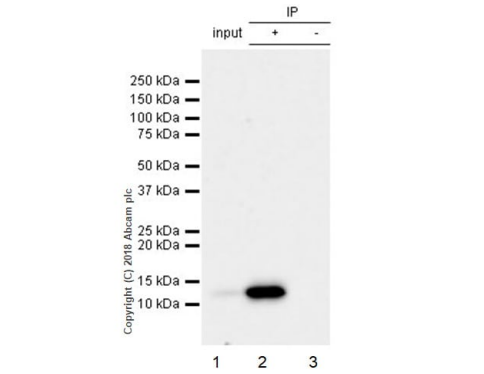
Immunocytochemistry/ Immunofluorescence - Anti-MCP1 antibody [EPR21025] (ab214819)

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

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)

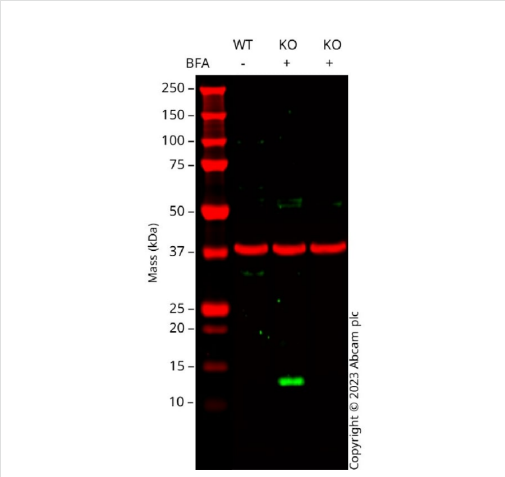
MCP1 was immunoprecipitated from 0.35 mg of THP-1 (human monocytic leukemia cell line) treated with 80nM Phorbol-12-myristate-13-acetate (PMA, **ab120297**) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate 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.



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

All lanes : Anti-MCP1 antibody [EPR21025] (ab214819) at 1/400 dilution

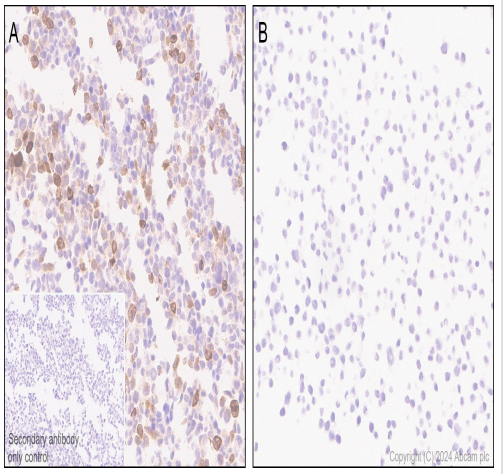
Lane 1 : Wild-type A549 cell lysate
Lane 2 : Wild-type A549 Treated BFA (1 ug/mL, 3 h) cell lysate
Lane 3 : CCL2 knockout A549 Treated BFA (1 ug/mL, 3 h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 11 kDa
Observed band size: 13 kDa

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.

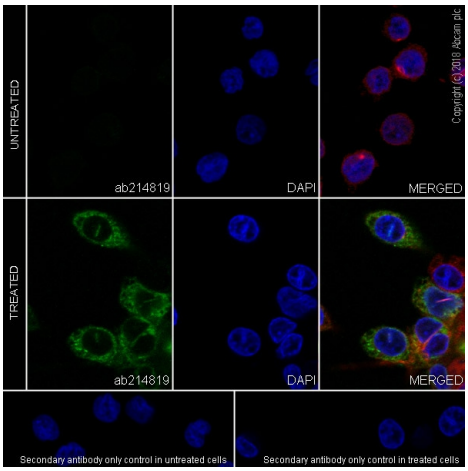


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] (ab214819)

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.

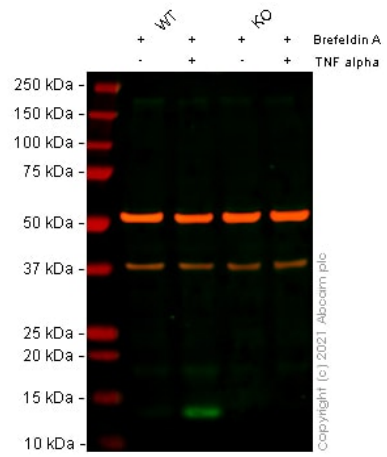


Immunocytochemistry/ Immunofluorescence - Anti-MCP1 antibody [EPR21025] (ab214819)

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.



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

All lanes : Anti-MCP1 antibody [EPR21025] (ab214819) at 1/1000 dilution

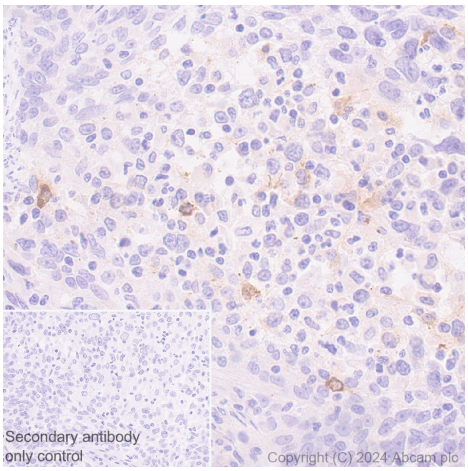
Lane 1 : Wild-type HeLa Vehicle Control TNF alpha (0ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate
Lane 2 : Wild-type HeLa Treated TNF alpha (20ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate
Lane 3 : CCL2 knockout HeLa Vehicle Control TNF alpha (0ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate
Lane 4 : CCL2 knockout HeLa Treated TNF alpha (20ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 11 kDa

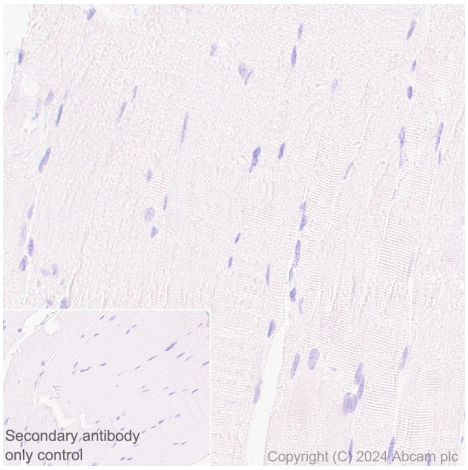
False colour image of Western blot: Anti-MCP1 antibody [EPR21025] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab214819 was shown to bind specifically to MCP1. A band was observed 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 y 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.



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.

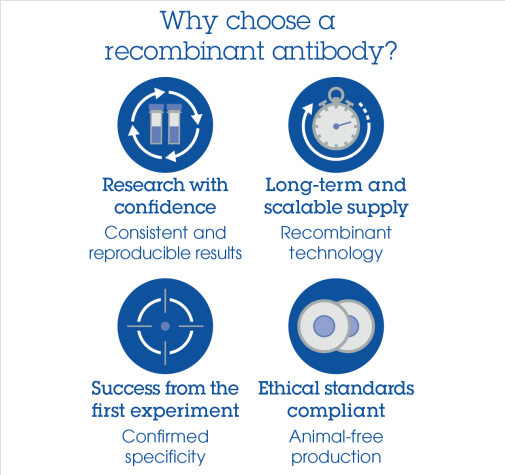


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] (ab214819)

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.



Anti-MCP1 antibody [EPR21025] (ab214819)

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