abcam

## Product datasheet

# Anti-MCP1 antibody [EPR21025] - BSA and Azide free ab242013

KO VALIDATED Recombinant RabMAb

**1 References** 11 Images

### Overview

| | |
|---|---|
| **Product name** | Anti-MCP1 antibody [EPR21025] - BSA and Azide free |
| **Description** | Rabbit monoclonal [EPR21025] to MCP1 - BSA and Azide free |
| **Host species** | Rabbit |
| **Specificity** | Stimulation may be required for the detection of MCP1, as it is not constitutively expressed. |
| **Tested applications** | **Suitable for:** Flow Cyt (Intra), ICC/IF, IHC-P, WB, IP |
| **Species reactivity** | **Reacts with:** Human |
| **Immunogen** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | ICC/IF: THP-1 treated with PMA , 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 for 24h, then treated with LPS for 4h, then together with BFA for 3h. IP: THP-1 treated with PMA for 24h, then treated with LPS for 4h, then together with BFA for 3h 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. |
| **General notes** | ab242013 is the carrier-free version of **ab214819**. |

Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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**.

## Properties

**Form** Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

**Storage buffer** pH: 7.2
Constituent: PBS

**Carrier free** Yes

**Purity** Protein A purified

**Clonality** Monoclonal

**Clone number** EPR21025

**Isotype** IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab242013 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| **Flow Cyt (Intra)** | | Use at an assay dependent concentration. |
| **ICC/IF** | | Use at an assay dependent concentration. |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **WB** | | Use at an assay dependent concentration. Detects a band of approximately 11 kDa (predicted molecular weight: 11 kDa). |
| **IP** | | Use at an assay dependent concentration. |

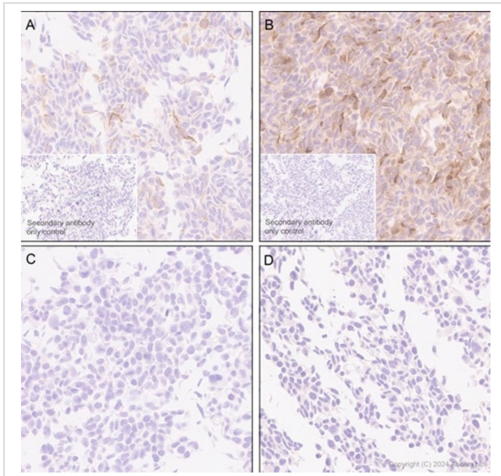
## Target

**Function** 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.

**Sequence similarities** Belongs to the intercrine beta (chemokine CC) family.

| | |
|---|---|
| **Post-translational modifications** | 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. |
| **Cellular localization** | Secreted. |

## Images



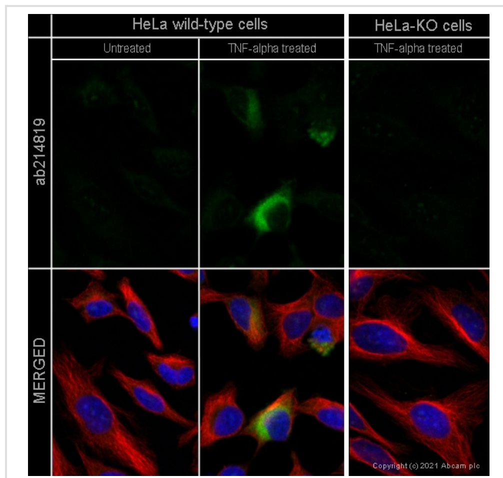
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

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.

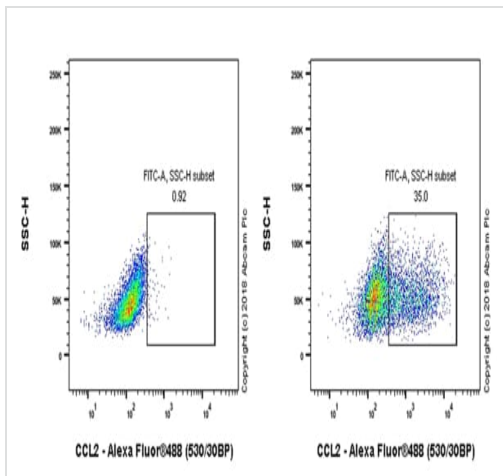
This data was developed using **ab214819**, the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide.




Immunocytochemistry/ Immunofluorescence - Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

This data was developed using the same antibody clone in a different buffer formulation (**ab214819**).

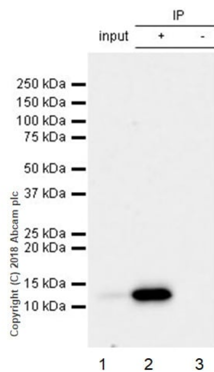
**ab214819** staining MCP1 in wild-type and MCP1 knockout HeLa cells (**ab255372**), untreated or treated with TNF-alpha (20ng/mL, 6 hours) and Brefeldin A (1µg/ml, 3 hours). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 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] - BSA and Azide free (ab242013)

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) 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab214819**).




Immunoprecipitation - Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

MCP1 was immunoprecipitated from 0.35 mg of THP-1 (human monocytic leukemia cell line) treated with 80nM Phorbol-12-myristate-13-acetate (PMA) 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 1/1000 dilution.

**Lane 1:** THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA) 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).

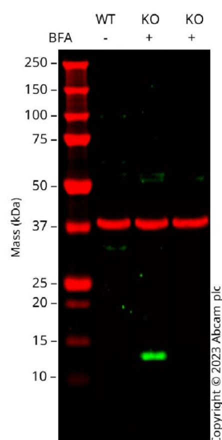
**Lane 2:** **ab214819** IP in THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab214819** in THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA) 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 and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab214819**).




Western blot - Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

**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,

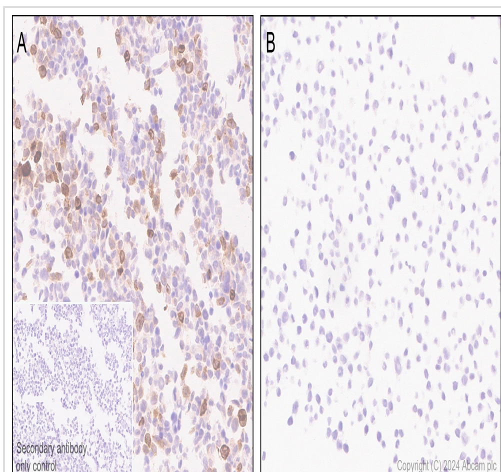
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab214819**).

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



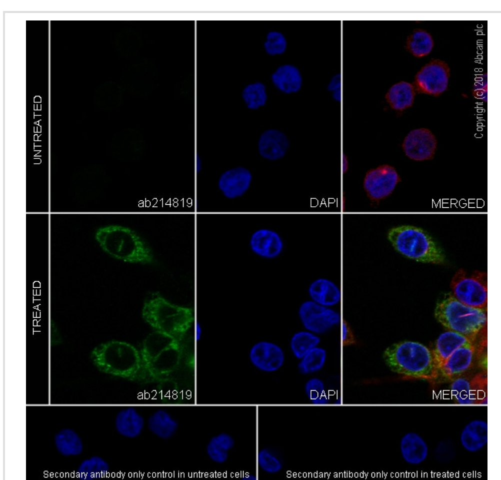

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

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.

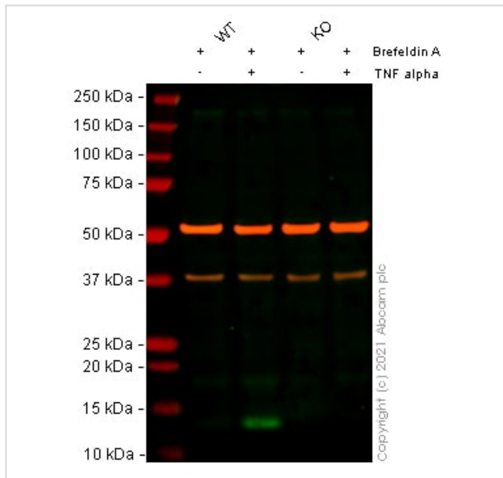
This data was developed using **ab214819**, the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide.




Immunocytochemistry/ Immunofluorescence - Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

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) 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab214819**).




Western blot - Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

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

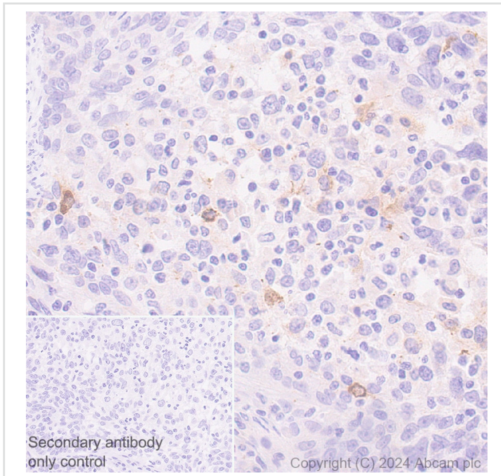
**Lane 1 :** Wild-type HeLa Vehicle Control TNF alpha (0ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate
**Lane 2 :** Wild-type HeLa Treated TNF alpha (20ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell lysate
**Lane 3 :** CCL2 knockout HeLa Vehicle Control TNF alpha (0ng/mL, 6h) + Brefeldin A (1µg/ml,3h) cell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

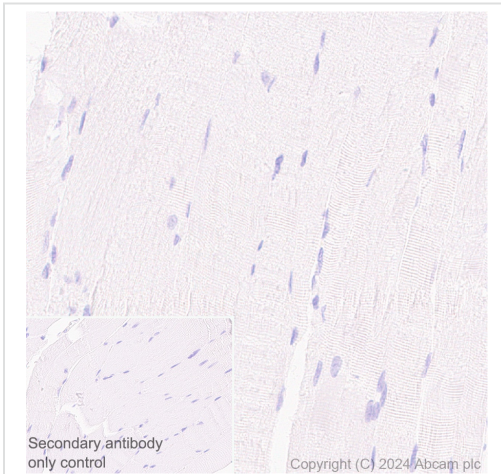



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

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.

This data was developed using **ab214819**, the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide.




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

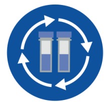
Immunohistochemical analysis of paraffin-embedded human skeletal muscle sections labelling MCP1 with **ab214819** at a 1/10000 dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) secondary antibody. Counter stained with Hematoxylin.

**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.

This data was developed using **ab214819**, the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide.

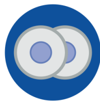
Why choose a recombinant antibody?



**Research with confidence**
Consistent and reproducible results

**Long-term and scalable supply**
Recombinant technology

**Success from the first experiment**
Confirmed specificity



**Ethical standards compliant**
Animal-free production

Anti-MCP1 antibody [EPR21025] - BSA and Azide free (ab242013)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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