# abcam

## Product datasheet

## Anti-Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 antibody [EPR23751-54] ab259372

Recombinant RabMAb

#### 1 References 13 Images

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**Product name** Anti-Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 antibody [EPR23751-54]

**Description** Rabbit monoclonal [EPR23751-54] to MIP-1 alpha/CCL3 + CCL3L1

**Host species** Rabbit

Suitable for: Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF **Tested applications** 

Species reactivity Reacts with: Mouse. Human

This product was produced with the following immunogens: **Immunogen** 

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

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Positive control WB: THP-1 (treated with PMA / LPS(Lipopolysaccharide / Brefeldin A) whole cell lysate;

> RAW264.7 (treated with LPS / Brefeldin A) whole cell lysate; Recombinant himan CCL3 protein; Recombinant human CCI3L1 protein. IHC-P: Human Hodgkin lymphoma tissue. ICC/IF: THP-1 and RAW264.7 cells (treated with PMA / LPS / Brefeldin A). Flow Cyt (intra): THP-1 and RAW264.7 cells (treated with PMA / LPS / Brefeldin A). IP: THP-1 (treated with PMA /

LPS(Lipopolysaccharide / Brefeldin A) whole cell lysate.

General notes 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** 

Storage instructions 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.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

ClonalityMonoclonalClone numberEPR23751-54

**Isotype** IgG

## **Applications**

**The Abpromise guarantee** Our <u>Abpromise guarantee</u> covers the use of ab259372 in the following tested applications.

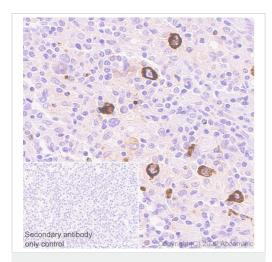
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 12 kDa (predicted molecular weight: 10 kDa).
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.
ICC/IF		1/50.

I	arget	

Cellular localization Macrophage Inflammatory Protein 1 alpha / CCL3: Secreted. CCL3L1: Secreted.

## **Images**

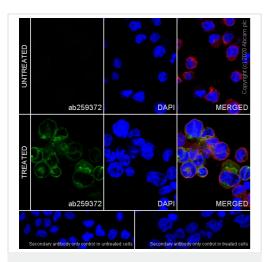


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Macrophage
Inflammatory Protein 1 alpha / CCL3 + CCL3L1
antibody [EPR23751-54] (ab259372)

Immunohistochemical analysis of paraffin-embedded human Hodgkin lymphoma tissue labeling Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 with ab259372 at 1/1000 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<a href="mailto:ab209101">ab209101</a>). Positive staining on human hodgkin Lymphoma (PMID: 31581676). The section was incubated with ab259372 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

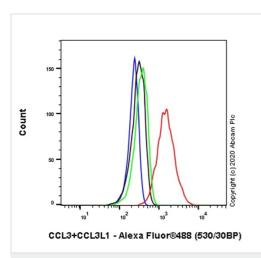
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 antibody [EPR23751-54] (ab259372)

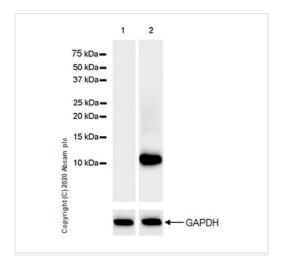
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X100 permeabilized THP-1 (human monocytic leukemia monocyte) cells labeling Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 with ab259372 at 1/100 dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in THP-1 cells treated with 12-myristate 13-acetate (100 nM) for 16 hours then with lipopolysaccharide (100 ng/ml) for 4 hours and with brefeldin A (1µg/ml) for another 3 hours. Tubulin is counterstained using ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) at 1/200 dilution (Red). The nuclear counterstain is DAPI (Blue).

Secondary antibody only control: Used PBS instead of priary antibody, followed by <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) secondary antibody at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 antibody [EPR23751-54] (ab259372)

Intracellular flow cytometric analysis of 5% paraformaldehyde-fixed, 90% methanol-permeabilized THP-1 (Human monocytic leukemia monocyte) (treated with 100nM phorbol 12-myristate 13-acetate (PMA) for 16 hours, then 100ng/ml lipopolysaccharide (LPS) for 4 hours, and add 1ug/ml Brefeldin A (BFA) for another 3 hours) (Red)/ Untreated control (Green) cells labeling Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 with ab259372 at 1/500 dilution (Red / Green) compared with a Isotype control details (ab172730) (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-Macrophage Inflammatory
Protein 1 alpha / CCL3 + CCL3L1 antibody
[EPR23751-54] (ab259372)

**All lanes :** Anti-Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 antibody [EPR23751-54] (ab259372) at 1/1000 dilution

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

**Lane 2 :** THP-1 (treated with 100nM PMA for 16 hours. And then replace PMA with 100 ng/ml LPS (Lipopolysaccharide) for 4 hours, and then 1  $\mu$ g/ml Brefeldin A was added for the last 3 hours), whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Predicted band size: 10 kDa Observed band size: 12 kDa Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 8 seconds.

Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 was immunoprecipitated from 0.35 mg THP-1 (human monocytic leukemia monocyte) (treated with 100nM PMA for 16 hours. And then replace PMA with 100 ng/ml LPS (Lipopolysaccharide) for 7 hours, 1 μg/ml BFA was added for the last 3 hours) whole cell lysate whole cell lysate with ab259372 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab259372 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: THP-1 (treated as above) whole cell lysate 3 ug

Lane 2: ab259372 IP in THP-1 (treated as above) whole cell lysate

 $\begin{tabular}{ll} \textbf{Lane 3:} & Rabbit monoclonal IgG ($\underline{ab172730}$) instead of ab259372 \\ & in THP-1 (treated as above) whole cell lysate \\ \end{tabular}$ 

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 8 seconds.

**All lanes :** Anti-Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 antibody [EPR23751-54] (ab259372) at 1/5000 dilution

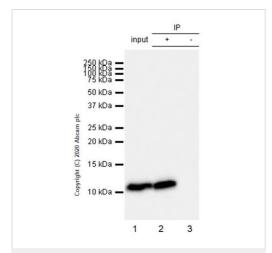
Lane 1: Untreated RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate Lane 2: RAW 264.7 (treated with 100 ng/ml LPS(Lipopolysaccharide) for 4 hours and then 1 µg/ml Brefeldin A was added for the last 3 hours), whole cell lysate

Lysates/proteins at 10 µg per lane.

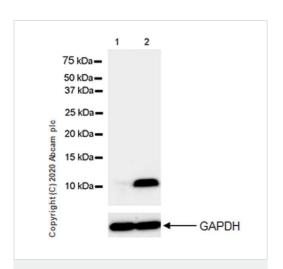
## Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 10 kDa **Observed band size:** 12 kDa



Immunoprecipitation - Anti-Macrophage Inflammatory
Protein 1 alpha / CCL3 + CCL3L1 antibody
[EPR23751-54] (ab259372)



Western blot - Anti-Macrophage Inflammatory
Protein 1 alpha / CCL3 + CCL3L1 antibody
[EPR23751-54] (ab259372)

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 seconds.

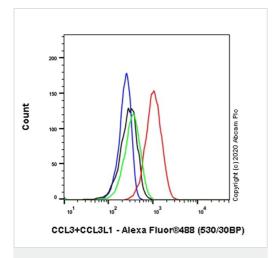
ab259372 DAPI MERGED

ab259372 DAPI MERGED

Secondary antibidity only control in untreated cells

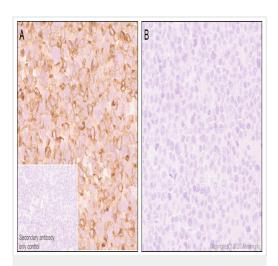
Immunocytochemistry/ Immunofluorescence - Anti-Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 antibody [EPR23751-54] (ab259372)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X100 permeabilized RAW264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cells labeling Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 with ab259372 at 1/100 dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in RAW 264.7 cells treated with 12-myristate 13-acetate (100 nM) for 16 hours then with lipopolysaccharide (100 ng/ml) for 4 hours and with brefeldin A (1µg/ml) for another 3 hours. Tubulin is counterstained using ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution (Red). The nuclear counterstain is DAPI (Blue). Secondary antibody only control: Used PBS instead of priary antibody, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) secondary antibody at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 antibody [EPR23751-54] (ab259372)

Intracellular flow cytometric analysis of5% paraformaldehyde-fixed, 90% methanol-permeabilized RAW264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) (treated with 100ng/ml lipopolysaccharide (LPS) for 4 hours, and add Brefeldin A (BFA) for another 3 hours (Red)/ Untreated control (Green)) (Red)/ Untreated control (Green) cells labeling Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 with ab259372at 1/500 dilution (Red / Green) compared with a Isotype control details (ab172730) (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor®488, ab150077), at 1/2000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Macrophage
Inflammatory Protein 1 alpha / CCL3 + CCL3L1
antibody [EPR23751-54] (ab259372)

Western blot - Anti-Macrophage Inflammatory
Protein 1 alpha / CCL3 + CCL3L1 antibody
[EPR23751-54] (ab259372)

Immunohistochemical analysis of paraffin-embedded THP-1 cells labeling Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 with ab259372 at 1/1000 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on THP-1 treated with 100nM PMA, LPS and Brefeldin treatment (image A), and negative staining on untreat THP-1 (image B). The section was incubated with ab259372 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

**All lanes :** Anti-Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 antibody [EPR23751-54] (ab259372) at 1/1000 dilution

**Lane 1 :** His-tagged mouse CCL3 recombinant protein (aa24-92) \* 2 ,10 ng

**Lane 2 :** GST/His-tagged human CCL3L1 recombinant protein (aa24-93), 10 ng

Lane 3: LIF/His-tagged human CCL4 recombinant protein (aa24-92)\*2, 10 ng

Lane 4: His-tagged human CCL18 recombinant protein (aa21-89),10 ng

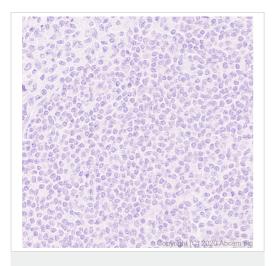
### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Predicted band size: 10 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposire time: 10 seconds.

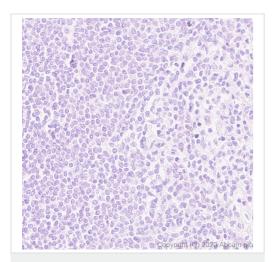


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Macrophage
Inflammatory Protein 1 alpha / CCL3 + CCL3L1
antibody [EPR23751-54] (ab259372)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 with ab259372 at 1/1000 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Negative control: No staining on human tonsil. The section was incubated with ab259372 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

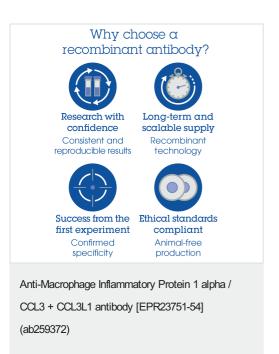


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Macrophage
Inflammatory Protein 1 alpha / CCL3 + CCL3L1
antibody [EPR23751-54] (ab259372)

Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling Macrophage Inflammatory Protein 1 alpha / CCL3 + CCL3L1 with ab259372 at 1/1000 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Negative control: No staining on human spleen tissue. The section was incubated with ab259372 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



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

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