# abcam

### Product datasheet

# Anti-CD14 antibody [EPR21847] ab221678

Recombinant RabMAb

8 References 8 Images

Overview

**Product name** Anti-CD14 antibody [EPR21847]

**Description** Rabbit monoclonal [EPR21847] to CD14

Host species Rabbit

Tested applications Suitable for: IHC-Fr, IP, Flow Cyt, ICC/IF, WB

Species reactivity Reacts with: Mouse

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: J774A.1 and RAW264.7 whole cell lysate; mouse lymph node and placenta lysates. ICC/IF:

J774A.1 and RAW 264.7 cells. Flow cyt: RAW 264.7 cells, C57 BL/6 mouse bone marrow cells.

IP: RAW 264.7 whole cell lysate; IHC-Fr: Mouse spleen tissue.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

Improved sensitivity and specificityLong-term security of supply

- Animal-free production

For more information **see here**.

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

**Properties** 

Form Liquid

**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** pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR21847

1

**Isotype** IgG

# **Applications**

The Abpromise guarantee

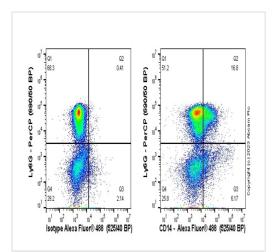
Our <u>Abpromise guarantee</u> covers the use of ab221678 in the following tested applications.

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

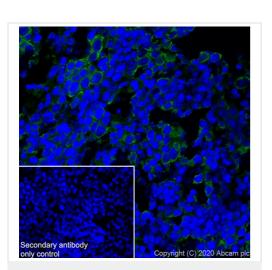
Application	Abreviews	Notes
IHC-Fr		Use at an assay dependent concentration.
IP		1/30.
Flow Cyt		1/500.
ICC/IF		1/1000.
WB		1/1000. Detects a band of approximately 50-55 kDa (predicted molecular weight: 39 kDa).

Target		
Function	Cooperates with MD-2 and TLR4 to mediate the innate immune response to bacterial lipopolysaccharide (LPS). Acts via MyD88, TIRAP and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response. Up-regulates cell surface molecules, including adhesion molecules.	
Tissue specificity	Expressed strongly on the surface of monocytes and weakly on the surface of granulocytes; also expressed by most tissue macrophages.	
Sequence similarities	Contains 11 LRR (leucine-rich) repeats.	
Post-translational modifications	N- and O- glycosylated. O-glycosylated with a core 1 or possibly core 8 glycan.	
Cellular localization	Cell membrane.	

## **Images**



Flow Cytometry - Anti-CD14 antibody [EPR21847] (ab221678)



Immunohistochemistry (Frozen sections) - Anti-CD14 antibody [EPR21847] (ab221678)

Flow cytometry staining of C57 BL/6 mouse bone marrow cells with ab221678 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). Cells were incubated for 30 min on ice in 1x PBS containing 10 $\mu$ g/ml anti CD16/CD32 and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab221678 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10<sup>6</sup> in 100  $\mu$ l at 10.0  $\mu$ g/ml (1/215)) for 30min on ice. The cells were simultaneously stained with Ly6G.

The secondary antibody Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min on ice

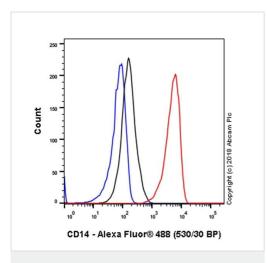
Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on

viable cells.

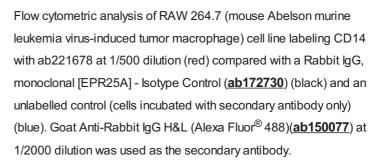
Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse spleen tissue labeling CD14 with ab221678 at 1/50 (10.62 ug/ml) dilution followed by **ab150077** Goat Anti-Rabbit lgG H&L (Alexa Fluor ® 488) at 1/1000 dilution (Green). Positive staining on mouse spleen. is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor ®; 488) at 1000 dilution.

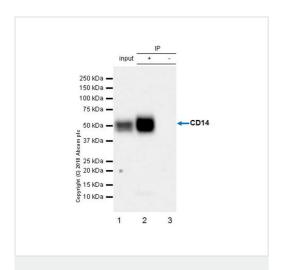
Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Flow Cytometry - Anti-CD14 antibody [EPR21847] (ab221678)



Gated on viable cells.



Immunoprecipitation - Anti-CD14 antibody [EPR21847] (ab221678)

CD14 was immunoprecipitated from 0.35 mg RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate with ab221678 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab221678 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

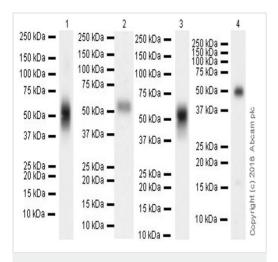
Lane 1: RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate 10 µg (Input).

Lane 2: ab221678 IP in RAW 264.7 whole cell lysate(+).

**Lane 3:** Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab221678 in RAW 264.7 whole cell lysate (-).

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 8 seconds

The molecular mass observed is consistent with the literature (PMID: 9502426; PMID:7513013)



Western blot - Anti-CD14 antibody [EPR21847] (ab221678)

**All lanes :** Anti-CD14 antibody [EPR21847] (ab221678) at 1/1000 dilution

**Lane 1 :** J774A.1 (mouse reticulum cell sarcoma monocyte macrophage), whole cell lysate at 10  $\mu g$ 

Lane 2: Mouse lymph node lysate at 20 µg

**Lane 3 :** RAW264.7 (mouse Abelson murine leukemia virusinduced tumor macrophage), whole cell lysate at 10  $\mu$ g

Lane 4: Mouse placenta lysate at 10 µg

#### Secondary

**Lanes 1-3 :** Goat Anti-Rabbit  $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$  at 1/100000 dilution

Lane 4 : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/50000 dilution

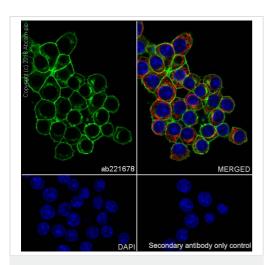
Developed using the ECL technique.

**Predicted band size:** 39 kDa **Observed band size:** 50-55 kDa

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

**Exposure time:** Lane 1: 6 seconds; Lane 2: 3 minutes; Lane 3: 10 seconds; Lane 4: 81 seconds.

The molecular mass observed is consistent with the literature (PMID: 9502426; PMID: 7513013).

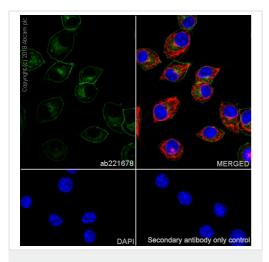


Immunocytochemistry/ Immunofluorescence - Anti-CD14 antibody [EPR21847] (ab221678)

Immunofluorescent analysis of 100% methanol-fixed RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labeling CD14 with ab221678 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous and cytoplasmic staining on RAW 264.7 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (<u>ab195889</u>) at 1/200 dilution (re

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

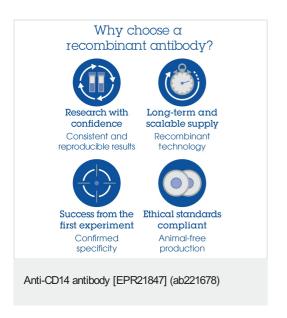


Immunocytochemistry/ Immunofluorescence - Anti-CD14 antibody [EPR21847] (ab221678)

Immunofluorescent analysis of 100% methanol-fixed J774A.1 (mouse reticulum cell sarcoma monocyte macrophage) cells labeling CD14 with ab221678 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous and cytoplasmic staining on J774A.1 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (<u>ab195889</u>) at 1/200 dilution (red).

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



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

#### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.com/abpromise">https://www.abcam.com/abpromise</a> or contact our technical team.

#### Terms and conditions

· Guarantee only valid for products bought direct from Abcam or one of our authorized distributors