# abcam

## Product datasheet

## Anti-CD14 antibody [EPR21847] ab221678



重组 RabMAb

8 References 8 图像

#### 概述

产品名称 Anti-CD14抗体[EPR21847]

描述 兔单克隆抗体[EPR21847] to CD14

宿主 Rabbit

经测试应用 适用于: IHC-Fr, IP, Flow Cyt, ICC/IF, WB

种属反应性 与反应: Mouse

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

阳性对照 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.

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  $\mathsf{RabMAb}^{\texttt{®}}$  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, 0.05% BSA

纯度 Protein A purified

克隆 单克隆

EPR21847 克隆编号

**同种型** IgG

## 应用

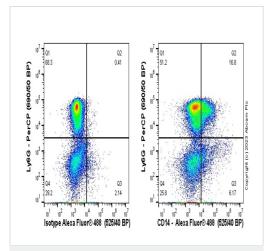
The Abpromise guarantee Abpromise™承诺保证使用ab221678于以下的经测试应用

"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
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).

靶标		
功能	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.	
组织 <b>特异性</b>	Expressed strongly on the surface of monocytes and weakly on the surface of granulocytes; also expressed by most tissue macrophages.	
序列相似性	Contains 11 LRR (leucine-rich) repeats.	
翻译后修饰	N- and O- glycosylated. O-glycosylated with a core 1 or possibly core 8 glycan.	
细胞定位	Cell membrane.	

图片

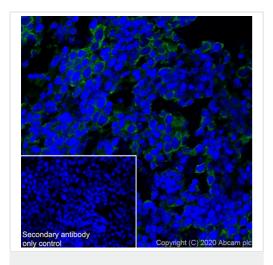


Flow Cytometry - Anti-CD14 antibody [EPR21847] (ab221678)

Flow cytometry staining of C57 BL/6 mouse bone marrow cells with ab221678 (right) or Recombinant Rabbit lgG, 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 lgG, 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.

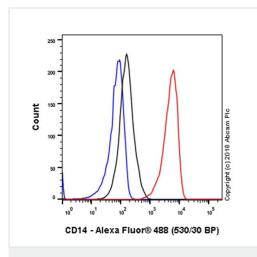


Immunohistochemistry (Frozen sections) - Anti-CD14 antibody [EPR21847] (ab221678)

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.

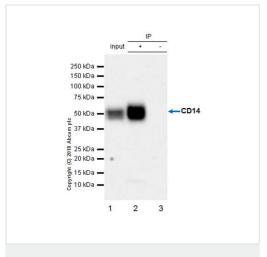
Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



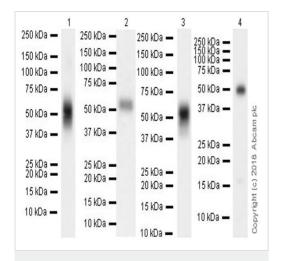
Flow Cytometry - Anti-CD14 antibody [EPR21847] (ab221678)

Flow cytometric analysis of RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cell line labeling CD14 with ab221678 at 1/500 dilution (red) compared with a Rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled control (cells incubated with secondary antibody only) (blue). Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488)(ab150077) at 1/2000 dilution was used as the secondary antibody.

Gated on viable cells.



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



Western blot - 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 lgG (<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)

**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 μg

Lane 2: Mouse lymph node lysate at 20 µg

**Lane 3**: RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate at 10 μg

Lane 4: Mouse placenta lysate at 10 µg

#### Secondary

Lanes 1-3: Goat Anti-Rabbit IgG H&L (HRP) (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

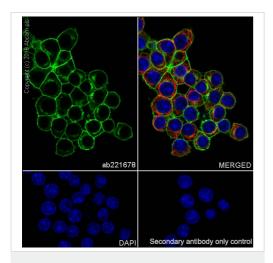
Observed band size: 50-55 kDa

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).

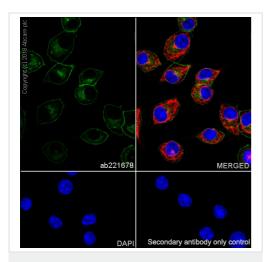


Immunocytochemistry/ Immunofluorescence - Anti-CD14 antibody [EPR21847] (ab221678)

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<sup>®</sup> 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 $^{\$}$  594) (ab195889) 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® 488) (ab150077) secondary at 1/1000 dilution.

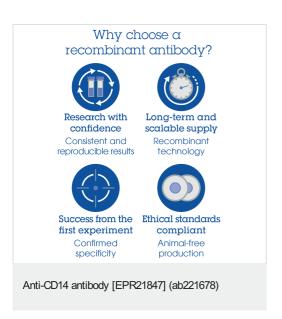


Immunocytochemistry/ Immunofluorescence - Anti-CD14 antibody [EPR21847] (ab221678)

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 lgG 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 lgG H&L (Alexa Fluor  $^{\$}$  488) (ab150077) secondary at 1/1000 dilution.



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