# abcam

# Product datasheet

# Anti-CD47 antibody [EPR24922-21] ab300124



יעלאעבני RabMAb

#### 画像数6

#### 製品の概要

製品名 Anti-CD47 antibody [EPR24922-21]

製品の詳細 Rabbit monoclonal [EPR24922-21] to CD47

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt, ICC/IF, WB

適用なし: IHC-P or IP

種交差性 交差種: Human

非交差種: Mouse, Rat

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

ポジティブ・コントロール WB: Jurkat, U937, A549 and NIH:OVCAR-3 lysates. ICC/IF: Jurkat and HEK293T cells. Flow cyt.:

Wild-type HEK-293T cell.

特記事項 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**.

#### 製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル クローン名 EPR24922-21

## アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab300124の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt		1/500.
ICC/IF		1/400.
WB		1/1000. Predicted molecular weight: 35 kDa.

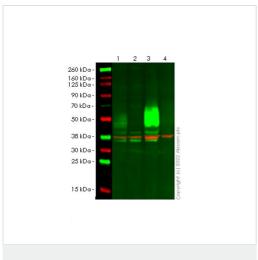
追加情報

Is unsuitable for IHC-P or IP.

## ターゲット情報

機能	Has a role in both cell adhesion by acting as an adhesion receptor for THBS1 on platelets, and in the modulation of integrins. Plays an important role in memory formation and synaptic plasticity in the hippocampus (By similarity). Receptor for SIRPA, binding to which prevents maturation of immature dendritic cells and inhibits cytokine production by mature dendritic cells. Interaction with SIRPG mediates cell-cell adhesion, enhances superantigen-dependent T-cell-mediated proliferation and costimulates T-cell activation. May play a role in membrane transport and/or integrin dependent signal transduction. May prevent premature elimination of red blood cells. May be involved in membrane permeability changes induced following virus infection.
組織特異性	Very broadly distributed on normal adult tissues, as well as ovarian tumors, being especially abundant in some epithelia and the brain.
配列類似性	Contains 1 lg-like V-type (immunoglobulin-like) domain.
細胞内局在	Cell membrane.

# 画像



Western blot - Anti-CD47 antibody [EPR24922-21] (AB300124)

**All lanes :** Anti-CD47 antibody [EPR24922-21] (ab300124) at 1/1000 dilution

**Lane 1 :** Wild-type HEK-293T (human embryonic kidney epithelial cell) whole cell lysate

Lane 2: CD47 knockout HEK-293T whole cell lysate

Lane 3 : Jurkat (human T cell leukemia T lymphocyte) whole cell lysate

**Lane 4 :** HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 35 kDa

Observed band size: 47-52 kDa

Blocking and diluting buffer and concentration: Intercept<sup>®</sup> (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS.

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Samples are non-boiled as boiling may cause protein aggregates. False colour image of Western blot: Anti-CD47 antibody [EPR24922-21] (ab300124) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab300124 was shown to bind specifically to CD47. A diffuse band was observed at 47-52 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in CD47 knockout cell line <a href="mailto:ab266324">ab266324</a> (knockout cell lysate <a href="mailto:ab257220">ab257220</a>). To generate this image, wild-type and CD47 knockout HEK-293T cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in Intercept<sup>®</sup> (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS 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 lgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (<u>ab216776</u>) at 1/20000 dilution.

Negative control: HepG2 (PMID: 28378740)

1 2 3 4 5

250 kDa—
150 kDa—
100 kDa—
75 kDa—
50 kDa—
50 kDa—
275 kDa—
20 kDa—

Western blot - Anti-CD47 antibody [EPR24922-21] (AB300124)

**All lanes :** Anti-CD47 antibody [EPR24922-21] (ab300124) at 1000 cells

**Lane 1 :** Jurkat (human T cell leukemia T lymphocyte) whole cell lysate

**Lane 2**: HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate

**Lane 3 :** U937 (human histiocytic lymphoma monocyte) whole cell lysate

**Lane 4**: A549 (human lung carcinoma epithelial cell) whole cell lysate

**Lane 5 :** NIH:OVCAR-3 (human ovary adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

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

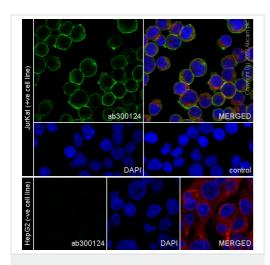
**Predicted band size:** 35 kDa **Observed band size:** 47-52 kDa

Blocking / Diluting buffer and concentration:

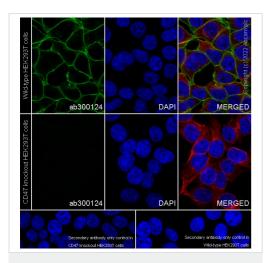
5% NFDM/TBST

Negative control: HepG2 (PMID: 28378740)

Samples are non-boiled as boiling may cause protein aggregates.



Immunocytochemistry/ Immunofluorescence - Anti-CD47 antibody [EPR24922-21] (ab300124)



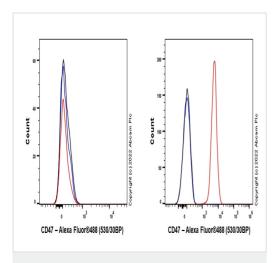
Immunocytochemistry/ Immunofluorescence - Anti-CD47 antibody [EPR24922-21] (ab300124)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeilized Jurkat (Human T cell leukemia T lymphocyte) cells lebelling CD47 with ab300124 at 1/100 (4.82 μg/ml) dilution, followed by ab150081 Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 μg/ml) dilution (Green). Confocal image showing membranous staining in Jurkat cell line. Negative control: HepG2 (PMID: 28378740). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5μg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

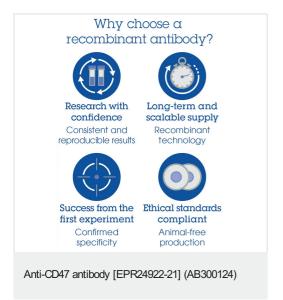
Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 (2  $\mu$ g/ml) dilution.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeilized CD47 KO HEK293T ( CD47 knockout human embryonic kidney epithelial cell) (ab266324) cells lebelling CD47 with ab300124 at 1/100 (4.82 μg/ml) dilution, followed by ab150081 Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed antibody at 1/1000 (2 μg/ml) dilution (Green). Confocal image showing membranous staining in Parental HEK293T cell line, no staining in CD47 KO HEK293T cell line is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 (2.5μg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 (2  $\mu$ g/ml) dilution.



Flow Cytometry - Anti-CD47 antibody [EPR24922-21] (AB300124) Flow cytometric analysis of Wild-type HEK-293T (human embryonic kidney epithelial cell, Right) / CD47 knockout HEK-293T (Left) cells labelling CD47 with ab300124 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells. Positive staining on HEK-293T cells (ab255449), while no staining on CD47 knockout HEK-293T cells (ab266324).



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