# abcam

## Product datasheet

## Anti-PHAPI2 / APRIL antibody [EPR14588] ab200836



ייבע RabMAb

**5 References** 画像数 14

#### 製品の概要

製品名 Anti-PHAPI2 / APRIL antibody [EPR14588]

製品の詳細 Rabbit monoclonal [EPR14588] to PHAPI2 / APRIL

由来種 Rabbit

アプリケーション 適用あり: IHC-P, WB, Flow Cyt (Intra), ICC/IF

種交差性 交差種: Mouse, Rat, Human

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

ポジティブ・コントロール WB: HEK293T, PC-3, LNCap, Jurkat, C6, RAW 264.7, PC-12, and NIH/3T3 whole cell lysates;

> Human fetal heart and fetal kidney lysates. IHC-P: Human prostatic hyperplasia, Human spleen, mouse spleen and rat cardiac muscle tissues. ICC/IF: Raw264.7 and Jurkat cells. Flow Cyt (intra):

Jurkat cells.

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

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 **EPR14588** 

## アプリケーション

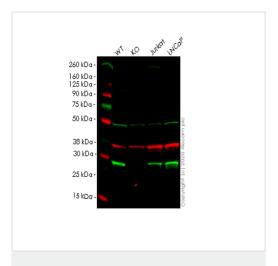
The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab200836の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 29 kDa (predicted molecular weight: 29 kDa).
Flow Cyt (Intra)		1/180.
ICC/IF		1/500.

## ターゲット情報

機能	Multifunctional protein working as a cell cycle progression factor as well as a cell survival factor. Required for the progression from the G1 to the S phase. Anti-apoptotic protein which functions as a caspase-3 inhibitor. Has no phosphatase 2A (PP2A) inhibitor activity (By similarity). Exhibits histone chaperone properties, stimulating core histones to assemble into a nucleosome.	
組織特異性	Expressed in heart, lung, pancreas, prostate and in spleen, thymus and placenta.	
配列類似性	Belongs to the ANP32 family.  Contains 4 LRR (leucine-rich) repeats.  Contains 1 LRRCT domain.	
ドメイン	Histone binding is mediated by the concave surface of the LRR region.	
翻訳後修飾	Some glutamate residues are glycylated by TTLL8. This modification occurs exclusively on glutamate residues and results in a glycine chain on the gamma-carboxyl group.	
細胞内局在	Nucleus. Accumulates in the nuclei at the S phase and Cytoplasm. Lacks a nuclear localization signal.	

#### 画像



Western blot - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

**All lanes :** Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: ANP32B knockout HEK293T cell lysate

Lane 3 : Jurkat cell lysate

Lane 4 : LNCaP cell lysate

Lysates/proteins at 20 µg per lane.

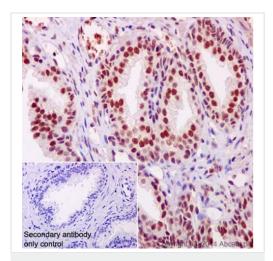
#### Secondary

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

Predicted band size: 29 kDa Observed band size: 29 kDa

**Lanes 1-4:** Merged signal (red and green). Green - ab200836 observed at 29 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab200836 Anti-PHAPI2 / APRIL antibody [EPR14588] was shown to specifically react with PHAPI2 / APRIL in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266818 (knockout cell lysate ab257831) was used. Wild-type and PHAPI2 / APRIL knockout samples were subjected to SDS-PAGE. ab200836 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

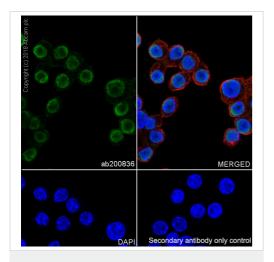


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

Immunohistochemical analysis of paraffin-embedded Human prostatic hyperplasia tissue labeling PHAPI2 / APRIL with ab200836 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear staining on Human prostatic hyperplasia tissue is observed. Counter stained with Hematoxylin.

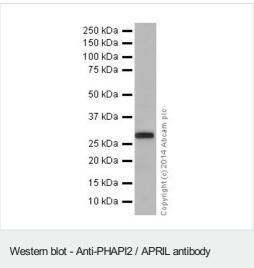
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) cells labeling PHAPI2 / APRIL with ab200836 at 1/250 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and Ab195889 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/200 dilution (red). Confocal image showing mostly nuclear staining in Raw264.7 cell line.



[EPR14588] (ab200836)

Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836) at 1/1000 dilution + PC-3 (Human prostate cancer cell line) whole cell lysate at 10 µg

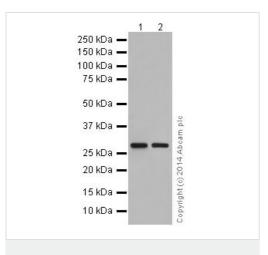
#### Secondary

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 29 kDa Observed band size: 29 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

All lanes: Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836) at 1/10000 dilution

Lane 1: LNCaP (Human prostate cancer cell line) whole cell lysate Lane 2: Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate

Lysates/proteins at 10 µg per lane.

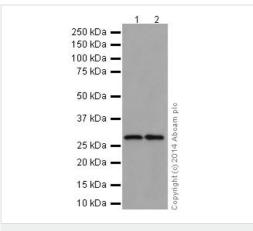
#### Secondary

All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 29 kDa Observed band size: 29 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PHAPI2 / APRIL antibody

[EPR14588] (ab200836)

All lanes: Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836) at 1/1000 dilution

Lane 1: Human fetal heart lysate Lane 2: Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

### Secondary

All lanes: Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 29 kDa Observed band size: 29 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

1 2 3 4 250 kDa • 150 kDa -100 kDa -75 kDa -50 kDa -Copyright (c) 2014 Abcam plo 37 kDa -25 kDa = 20 kDa -15 kDa -10 kDa -

Western blot - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

All lanes: Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836) at 1/1000 dilution

Lane 1: C6 (Rat glial tumor cells) whole cell lysate

Lane 2: RAW 264.7 (Mouse macrophage cells transformed with

Abelson murine leukemia virus) whole cell lysate

Lane 3: PC-12 (Rat adrenal gland pheochromocytoma) whole cell

lysate

Lane 4: NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes: Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 29 kDa Observed band size: 29 kDa Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

1 2 3
250 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —
10 kDa —
PHAPI2/APRIL peptide - + PHAPI peptide - + PHAPI peptide - + -

Western blot - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

**All lanes :** Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836) at 1/1000 dilution

Lane 1 : PC-3 (Human prostate cancer cell line) whole cell lysate

Lane 2 : PC-3 (Human prostate cancer cell line) whole cell lysate

with PHAPI2 / APRIL peptide

Lane 3: PC-3 (Human prostate cancer cell line) whole cell lysate with PHAPI peptide

Lysates/proteins at 10 µg per lane.

#### **Secondary**

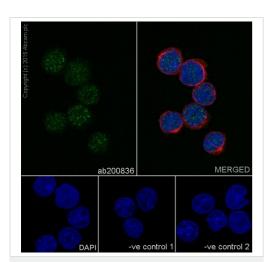
**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 29 kDa **Observed band size:** 29 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.

Based on sequence analysis ab 2008 36 has 78% homology with PHAPI protein. The levels of XR were tested in the accompanying blocking experiment.

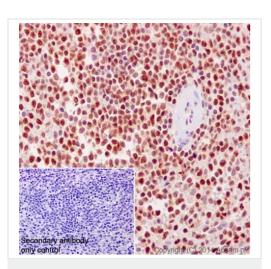


Immunocytochemistry/ Immunofluorescence - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling PHAPI2 / APRIL with ab200836 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Nuclear and cytoplasmic staining on Jurkat cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows;

-ve control 1: ab200836 at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.

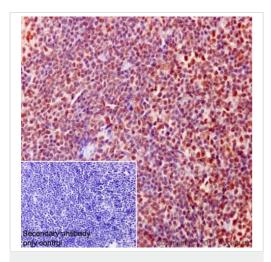


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling PHAPI2 / APRIL with ab200836 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear staining on Human spleen tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

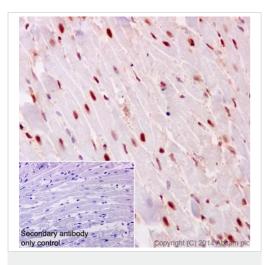


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling PHAPI2 / APRIL with ab200836 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear staining on Mouse spleen tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

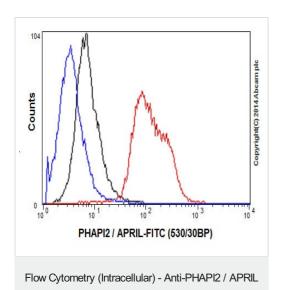


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PHAPI2 / APRIL antibody [EPR14588] (ab200836)

Immunohistochemical analysis of paraffin-embedded Rat cardiac muscle tissue labeling PHAPI2 / APRIL with ab200836 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear staining on Rat cardiac muscle tissue is observed. Counter stained with Hematoxylin.

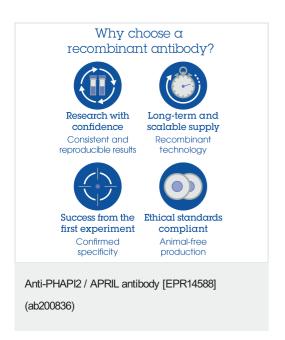
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



antibody [EPR14588] (ab200836)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling PHAPI2 / APRIL with ab200836 at 1/180 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730;black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.



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