abcam

Product datasheet

Anti-Rb antibody [EPR17512] ab181616

KO 評価済 リコンビナント RabMAb

70 References 画像数 13

製品の概要

製品名	Anti-Rb antibody [EPR17512]
製品の詳細	Rabbit monoclonal [EPR17512] to Rb
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), WB, IP, ICC/IF, IHC-P
種交差性	**交差種:** Mouse, Human, African green monkey
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, Hek294, K562, WEHI-3, COS-1, MCF7 and F9 whole cell lysates; Mouse brain and lung lysates; Human fetal brain lysate. IHC-P: Human lung, Human breast cancer, Mouse lung and Mouse cerebral cortex tissues. ICC/IF: MCF7 cells. IP: MCF7 whole cell lysate.
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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 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	EPR17512
アイソタイプ	IgG

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ab181616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 (Intra)		Use at an assay dependent concentration.
WB		1/2000. Detects a band of approximately 105 kDa (predicted molecular weight: 105 kDa). For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy).**
IP		1/80.
ICC/IF		1/500.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

ターゲット情報

機能 Key regulator of entry into cell division that acts as a tumor suppressor. Promotes G0-G1 transition when phosphorylated by CDK3/cyclin-C. Acts as a transcription repressor of E2F1 target genes. The underphosphorylated, active form of RB1 interacts with E2F1 and represses its transcription activity, leading to cell cycle arrest. Directly involved in heterochromatin formation by maintaining overall chromatin structure and, in particular, that of constitutive heterochromatin by stabilizing histone methylation. Recruits and targets histone methyltransferases SUV39H1, KMT5B and KMT5C, leading to epigenetic transcriptional repression. Controls histone H4 'Lys-20' trimethylation. Inhibits the intrinsic kinase activity of TAF1. Mediates transcriptional repression by SMARCA4/BRG1 by recruiting a histone deacetylase (HDAC) complex to the c-FOS promoter. In resting neurons, transcription of the c-FOS promoter is inhibited by BRG1-dependent recruitment of a phospho-RB1-HDAC1 repressor complex. Upon calcium influx, RB1 is dephosphorylated by calcineurin, which leads to release of the repressor complex (By similarity). In case of viral infections, interactions with SV40 large T antigen, HPV E7 protein or adenovirus E1A protein induce the disassembly of RB1-E2F1 complex thereby disrupting RB1's activity.

組織特異性 Expressed in the retina.

関連疾患 Childhood cancer retinoblastoma
Bladder cancer
Osteogenic sarcoma

配列類似性 Belongs to the retinoblastoma protein (RB) family.

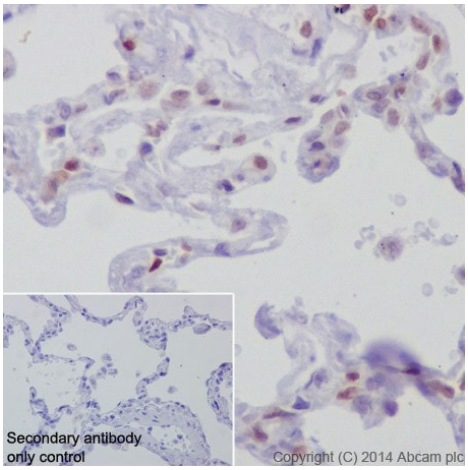
ドメイン The Pocket domain binds to the threonine-phosphorylated domain C, thereby preventing interaction with heterodimeric E2F/DP transcription factor complexes.

翻訳後修飾 Phosphorylated by CDK6 and CDK4, and subsequently by CDK2 at Ser-567 in G1, thereby releasing E2F1 which is then able to activate cell growth. Dephosphorylated at the late M phase. SV40 large T antigen, HPV E7 and adenovirus E1A bind to the underphosphorylated, active form of pRb. Phosphorylation at Thr-821 and Thr-826 promotes interaction between the C-terminal

domain C and the Pocket domain, and thereby inhibits interactions with heterodimeric E2F/DP transcription factor complexes. Dephosphorylated at Ser-795 by calcineruin upon calcium stimulation. CDK3/cyclin-C-mediated phosphorylation at Ser-807 and Ser-811 is required for G0-G1 transition. Phosphorylated by CDK1 and CDK2 upon TGFB1-mediated apoptosis.
N-terminus is methylated by METTL11A/NTM1 (By similarity). Monomethylation at Lys-810 by SMYD2 enhances phosphorylation at Ser-807 and Ser-811, and promotes cell cycle progression. Monomethylation at Lys-860 by SMYD2 promotes interaction with L3MBTL1.
Acetylation at Lys-873 and Lys-874 regulates subcellular localization, at least during keratinocytes differentiation.

細胞内局在 Nucleus.

画像

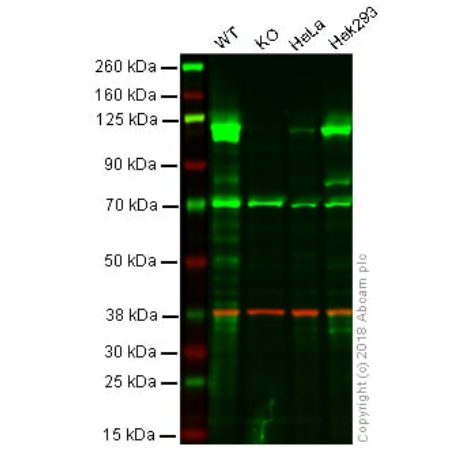


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rb antibody [EPR17512] (ab181616)

Immunohistochemical analysis of paraffin-embedded Human lung tissue labeling Rb with ab181616 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nuclear staining on Human lung is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.



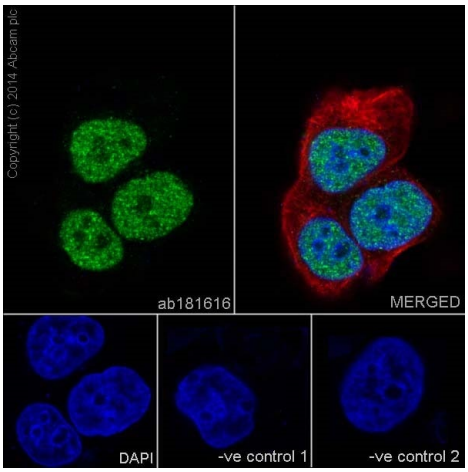
Western blot - Anti-Rb antibody [EPR17512] (ab181616)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: Rb knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: HEK293 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab181616 observed at 120 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

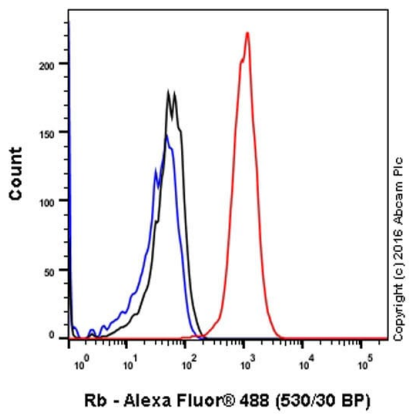
ab181616 was shown to recognize Rb in wild-type HAP1 cells as signal was lost at the expected MW in Rb knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and RB1 knockout samples were subjected to SDS-PAGE. Ab181616 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-

Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Rb antibody [EPR17512] (ab181616)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MCF7 (Human breast adenocarcinoma cell line) cells labeling Rb with ab181616 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green). Confocal image showing nuclear staining on MCF7 cells. 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: ab181616 at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

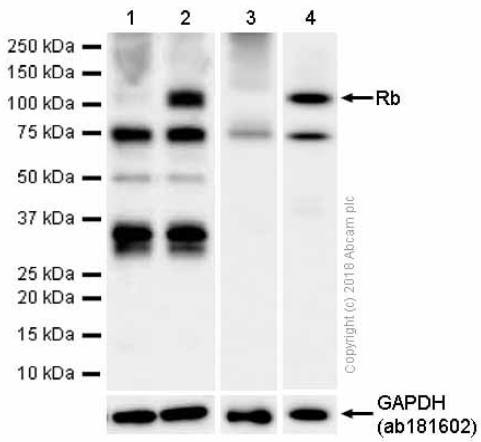


Flow Cytometry (Intracellular) - Anti-Rb antibody [EPR17512] (ab181616)

ab181616 staining Rbin the human cell line MCF-7 (human breast carcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde, permeabilized with 90% methanol and the sample was incubated with the primary antibody at a dilution of 1/140. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Western blot - Anti-Rb antibody [EPR17512] (ab181616)

All lanes : Anti-Rb antibody [EPR17512] (ab181616) at 1/1000 dilution

Lane 1 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates prepared in RIPA lysis method with 5% NFDM/TBST
Lane 2 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates prepared in 1%SDS Hot lysis method with 5% NFDM/TBST
Lane 3 : K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates prepared in RIPA lysis method with 5% NFDM/TBST
Lane 4 : K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates prepared in 1%SDS Hot lysis method with 5% NFDM/TBST

Lysates/proteins at 20 µg per lane.

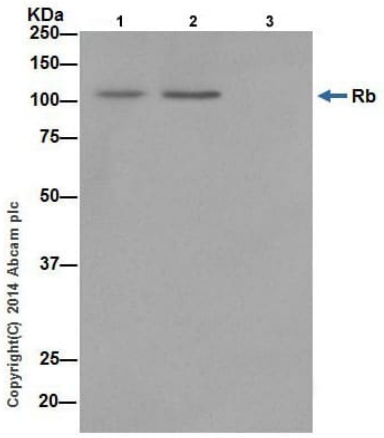
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

Predicted band size: 105 kDa

Exposure time:

Lane 1 and 2: 100 seconds
Lane 3 and 4: 10 seconds

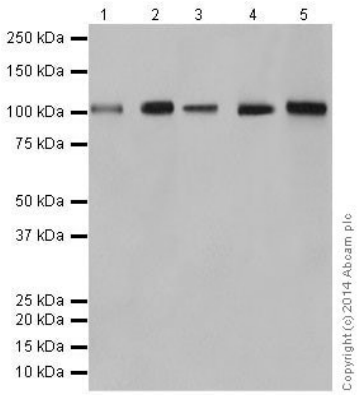
We recommend to use 1%SDS Hot lysis method to get clear band.
We are unsure how to define the extra bands.



Immunoprecipitation - Anti-Rb antibody [EPR17512] (ab181616)

Rb was immunoprecipitated from 1mg of MCF7 (Human breast adenocarcinoma cell line) whole cell lysate with ab181616 at 1/80 dilution. Western blot was performed from the immunoprecipitate using ab181616 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: MCF7 whole cell lysate 10 µg (Input). Lane 2: ab181616 IP in MCF7 whole cell lysate. Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab181616 in MCF7 whole cell lysate. Blocking and dilution buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-Rb antibody [EPR17512] (ab181616)

All lanes : Anti-Rb antibody [EPR17512] (ab181616) at 1/20000 dilution

Lane 1 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate
Lane 2 : K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate
Lane 3 : WEHI-3 (Mouse leukemia) whole cell lysate
Lane 4 : COS-1 (African green monkey kidney fibroblast-like cell line) whole cell lysate
Lane 5 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

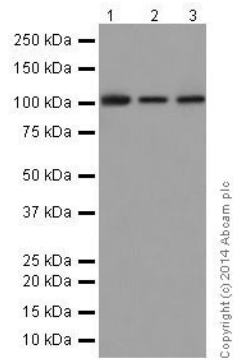
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 105 kDa
Observed band size: 105 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The lysates were all prepared using 1%SDS Hot lysis method.



Western blot - Anti-Rb antibody [EPR17512] (ab181616)

All lanes : Anti-Rb antibody [EPR17512] (ab181616) at 1/10000 dilution

Lane 1 : F9 (Mouse embyro testicular cancer cell line) whole cell lysate
Lane 2 : Mouse brain lysate
Lane 3 : Mouse lung lysate

Lysates/proteins at 10 µg per lane.

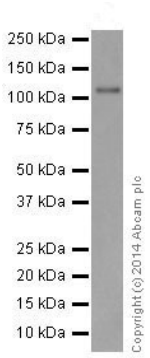
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 105 kDa
Observed band size: 105 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.

The lysates were all prepared using 1%SDS Hot lysis method.



Western blot - Anti-Rb antibody [EPR17512] (ab181616)

Anti-Rb antibody [EPR17512] (ab181616) at 1/2000 dilution + Human fetal brain lysate at 10 µg

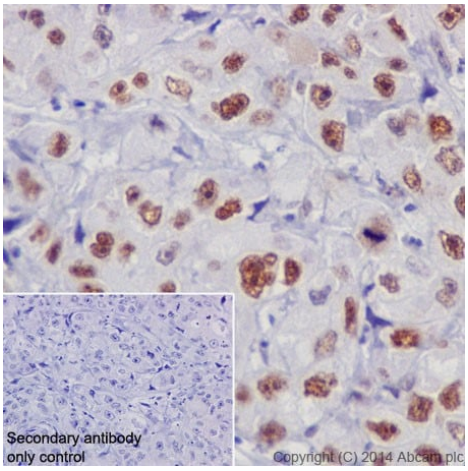
Secondary
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 105 kDa
Observed band size: 105 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.

The lysates were all prepared using 1%SDS Hot lysis method.

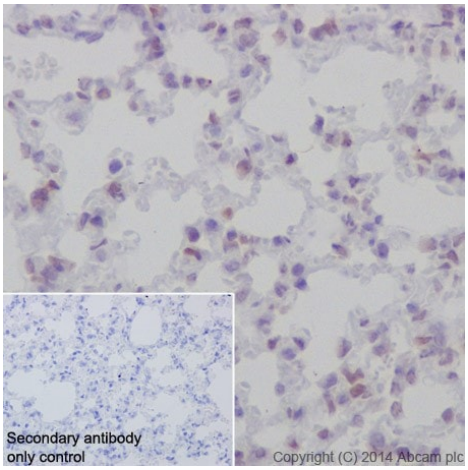


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rb antibody [EPR17512] (ab181616)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling Rb with ab181616 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nuclear staining on cancer cells of Human breast cancer is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.

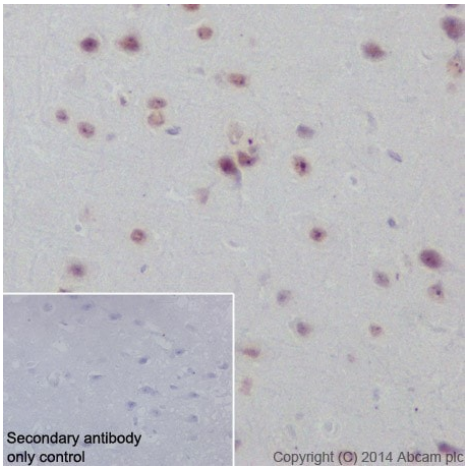


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rb antibody [EPR17512] (ab181616)

Immunohistochemical analysis of paraffin-embedded Mouse lung tissue labeling Rb with ab181616 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nuclear staining on mouse lung is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.

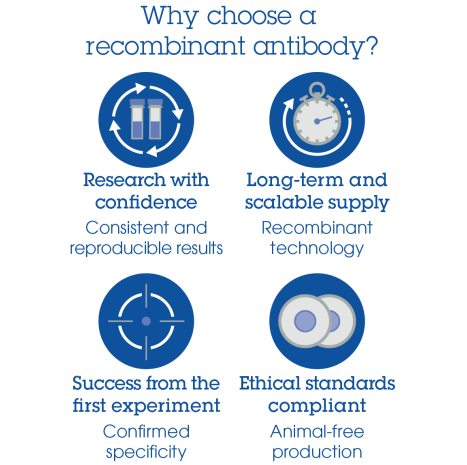


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rb antibody [EPR17512] (ab181616)

Immunohistochemical analysis of paraffin-embedded Mouse cerebral cortex tissue labeling Rb with ab181616 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nuclear staining on neuron of mouse cerebral cortex is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.



Anti-Rb antibody [EPR17512] (ab181616)

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