abcam

Product datasheet

Anti-Rb (phospho T373) antibody [EP821Y] ab52975

RabMAb

8 References　画像数 7

製品の概要

製品名	Anti-Rb (phospho T373) antibody [EP821Y]
製品の詳細	Rabbit monoclonal [EP821Y] to Rb (phospho T373)
由来種	Rabbit
アプリケーション	**適用あり:** WB, IP, IHC-P, ICC/IF, Dot blot **適用なし:** Flow Cyt
種交差性	**交差種:** 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	Jurkat nuclear cell lysate Human kidney carcinoma tissue
特記事項	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
バッファー	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
精製度	Protein A purified

ポリ/モノ	モノクローナル
クローン名	EP821Y
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ab52975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
WB		1/50000. Detects a band of approximately 110 kDa (predicted molecular weight: 106 kDa).
IP		1/30.
IHC-P		1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF		1/50 - 1/100.
Dot blot		1/1000 - 1/100000.

追加情報 Is unsuitable for Flow Cyt.

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.

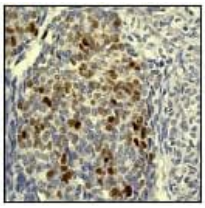
翻訳後修飾

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.

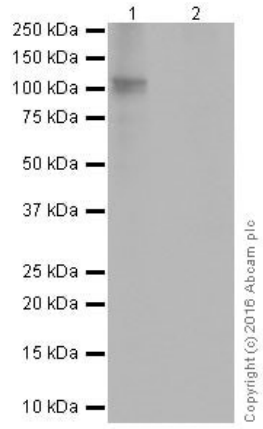
画像



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rb (phospho T373) antibody [EP821Y] (ab52975)

ab52975, at a 1/250 dilution, staining Human Rb (Phosphorylated) in Kidney carcinoma, using Immunohistochemistry, Paraffin Embedded tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-Rb (phospho T373) antibody [EP821Y] (ab52975)

All lanes : Anti-Rb (phospho T373) antibody [EP821Y] (ab52975) at 1/10000 dilution

Lane 1 : Jurkat (Human T cell leukemia T lymphocyte) serum starved for 24 h. Then 10% FBS incubated for 20 h. Whole cell lysates.
Lane 2 : Jurkat (Human T cell leukemia T lymphocyte) serum starved for 24 h. Then 10% FBS incubated for 20 h.

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

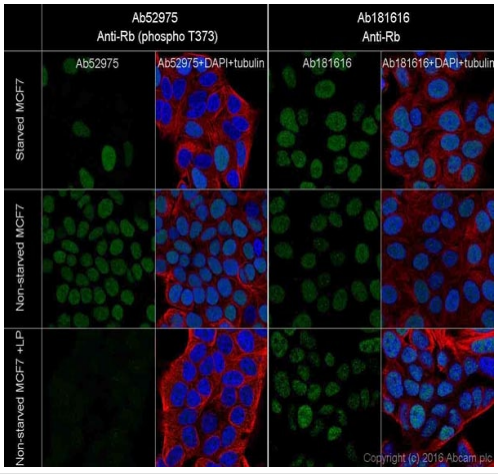
Predicted band size: 106 kDa
Additional bands at: 110 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 1 minute

Blocking and diluting buffer: 5% NFDM/TBST

The different result is due to the lysates preparation method. The lysate in lane 1 is prepared by 1%SDS Hot Lysate buffer method. The lysate in lane 2 is prepared by RIPA lysis method. This antibody works in 1%SDS Hot Lysates in WB.

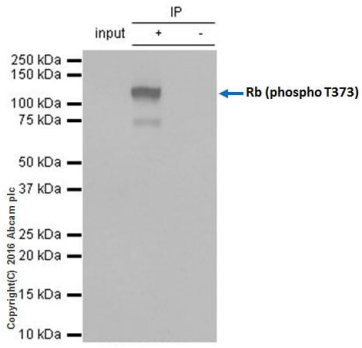
For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy).**



Immunocytochemistry/ Immunofluorescence - Anti-Rb (phospho T373) antibody [EP821Y] (ab52975)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% tritonX-100 permeabilized MCF7(Human breast denocarcinoma cell line) cells, Serum starved and non-starved, labeling Rb (phospho T373) with Ab52975 at 1/100 dilution followed by Goat anti-Rabbit secondary IgG AlexaFluor®488 (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on MCF7 cells.The number of positive cells increased after treatment with FBS (fetal bovine serum) for 48 hours, then decreased after Lambda Protein Phosphatase treatment (31 for 2hours).



Immunoprecipitation - Anti-Rb (phospho T373) antibody [EP821Y] (ab52975)

All lanes : Anti-Rb (phospho T373) antibody [EP821Y] (ab52975) at 1/500 dilution

Lane 1 : Jurkat (human acute T cell leukemia) starved 24h then 10% FBS incubated for 20h whole cell lysate.
Lane 2 : Jurkat (human acute T cell leukemia) starved 24h then 10% FBS incubated for 20h whole cell lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab52975 in Jurkat (human acute T cell leukemia) starved 24h then 10% FBS incubated for 20h whole cell lysate.

Lysates/proteins at 10 µg per lane.

Secondary
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/1000

dilution

Predicted band size : 106 kDa
Additional bands at : 110 kDa.

Exposure time : 1 second
Blocking and diluting buffer: 5% NFDM/TBST
The IP lysates are prepared by RIPA method. As the WB image shows, this antibody works in 1%SDS Hot Lysate buffer in WB. So there is no band in input lane.

For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy).**

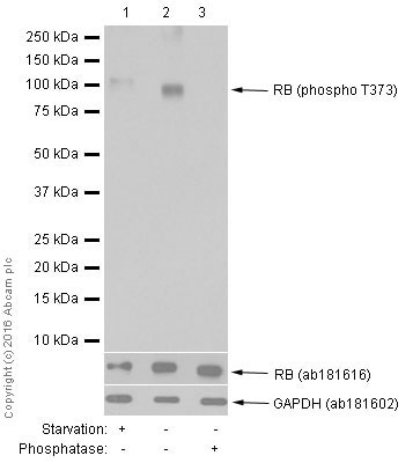
Lanes 1-3 : Anti-Rb (phospho T373) antibody [EP821Y] (ab52975) at 1/500 dilution

Lane 1 : Jurkat (human acute T cell leukemia) starved 24h then 10% FBS incubated for 20h whole cell lysate. at 10 µg
Lane 3 : Rabbit monoclonal IgG (**ab172730**) instead of ab52975 in Jurkat (human acute T cell leukemia) starved 24h then 10% FBS incubated for 20h whole cell lysate. at 10 µg

Secondary
Lanes 1-3 : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/1000 dilution

Additional bands at: 110 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 1 second



Western blot - Anti-Rb (phospho T373) antibody [EP821Y] (ab52975)

All lanes : Anti-Rb (phospho T373) antibody [EP821Y] (ab52975) at 1/10000 dilution

Lane 1 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) 2 day serum starved whole cell lysate.
Lane 2 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate.
Lane 3 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate. Membrane incubated with phosphatase.

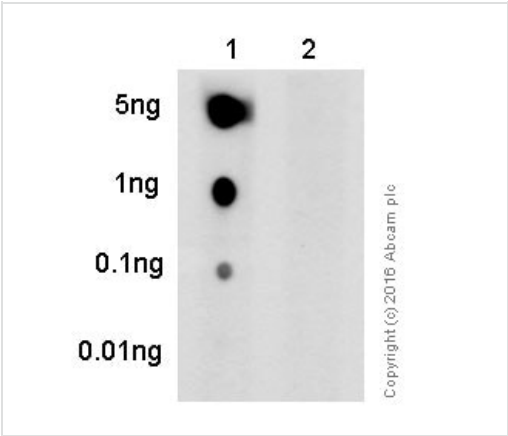
Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 106 kDa
Observed band size: 110 kDa

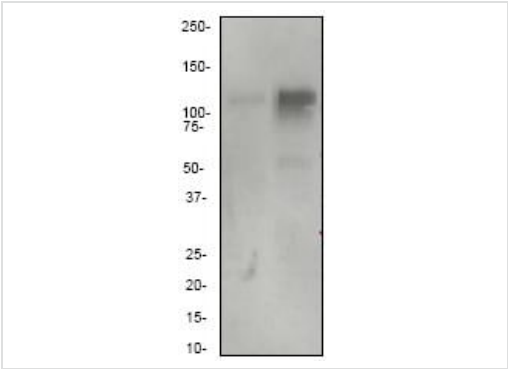
Exposure time: 30 seconds

The lysate in this image is prepared by 1%SDS Hot Lysate buffer.
For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy).**



Dot Blot - Anti-Rb (phospho T373) antibody [EP821Y] (ab52975)

Dot Blot analysis of Lane 1: Rb (pT373) phospho peptide and Lane 2: Rb non-phospho peptide labeling Rb (phospho T373) with ab52975 at 1/1000 dilution. 5% NFDM/TBST was used as the diluting and blocking buffer. **ab97051** Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated was used as the secondary antibody at 1/100000 dilution. Exposure time: 3 minutes.



Western blot - Anti-Rb (phospho T373) antibody [EP821Y] (ab52975)

All lanes : Anti-Rb (phospho T373) antibody [EP821Y] (ab52975) at 1/50000 dilution

Lane 1 : Jurkat cell lysate (untreated)
Lane 2 : Jurkat cell lysate (treated with FBS)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : goat anti-rabbit HRP labelled at 1/2000 dilution

Predicted band size: 106 kDa
Observed band size: 110 kDa

The lysate in this image is prepared by 1%SDS Hot Lysate buffer.

For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy).**

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