abcam

Product datasheet

Anti-Rb antibody [E182] ab32513

KO 評価済 リコンビナント RabMAb

3 References 画像数 6

製品の概要

製品名	Anti-Rb antibody [E182]
製品の詳細	Rabbit monoclonal [E182] to Rb
由来種	Rabbit
特異性	The antibody detects pan Rb protein.
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, IP, WB **適用なし:** IHC
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	WB: Hek293, and Jurkat cell lysates; ICC/IF: A431 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**. 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. 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: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
精製度	Protein A purified

ポリ/モノ	モノクローナル
クローン名	E182
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ab32513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)		1/70.
ICC/IF		1/50.
IP		1/40.
WB		1/1000. Detects a band of approximately 110 kDa (predicted molecular weight: 106 kDa).

追加情報 Is unsuitable for IHC.

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.

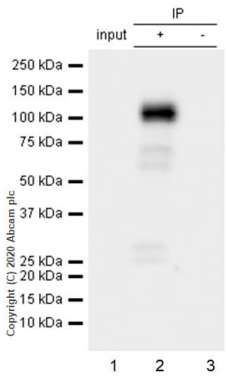
ドメイン The Pocket domain binds to the threonine-phosphorylated domain C, thereby preventing interaction with heterodimeric E2F/DP transcription factor complexes.

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.

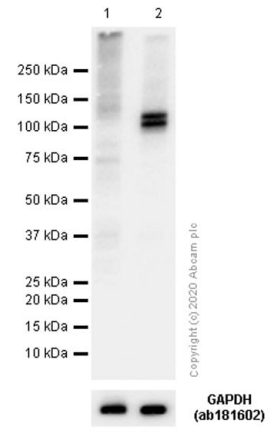
細胞内局在 Nucleus.

画像



Immunoprecipitation - Anti-Rb antibody [E182] (ab32513)

Purified ab32513 at 1/40 dilution (2µg) immunoprecipitating Rb in Jurkat whole cell lysate.
Lane 1 (input): Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10µg
Lane 2 (+): ab32513 + Jurkat whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32513 in Jurkat whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 120 kDa



Western blot - Anti-Rb antibody [E182] (ab32513)

All lanes : Anti-Rb antibody [E182] (ab32513) at 1/1000 dilution (Purified)

Lane 1 : Jurkat (Human T cell leukemia T lymphocyte) prepared in RIPA lysis method whole cell lysate
Lane 2 : Jurkat (Human T cell leukemia T lymphocyte) prepared in 1% SDS Hot lysis method whole cell lysate

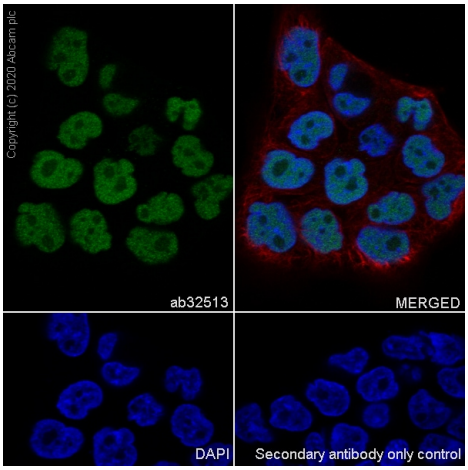
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 106 kDa
Observed band size: 106-120 kDa

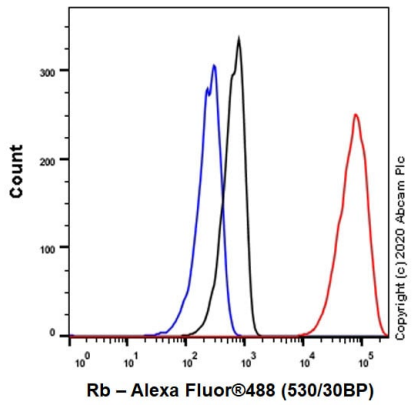
1% SDS Hot lysis method is preferred for this antibody.

Blocking Buffer and concentration: 5% NFDM/TBST



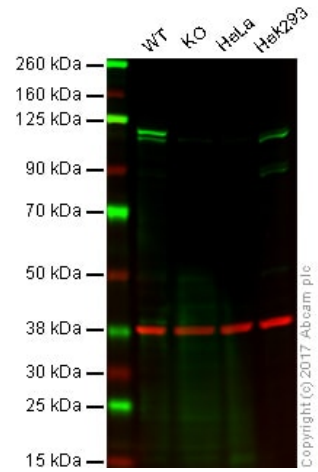
Immunocytochemistry/ Immunofluorescence - Anti-Rb antibody [E182] (ab32513)

Immunocytochemistry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling Rb with Purified ab32513 at 1:50 dilution (10 ?g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-Rb antibody [E182] (ab32513)

Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling Rb with Purified ab32513 at 1/70 dilution (10µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-Rb antibody [E182] (ab32513)

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 - ab32513 observed at 120 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32513 was shown to recognize Rb when Rb knockout samples were used, along with additional cross-reactive bands. Wild-type and Rb knockout samples were subjected to SDS-PAGE. Ab32513 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 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.

Anti-Rb antibody [E182] (ab32513)

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