abcam

Product datasheet

# Anti-Rb (phospho S780) antibody [EPR17624] ab184702

Recombinant RabMAb

2 References 7 Images

## Overview

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|---|---|
| **Product name** | Anti-Rb (phospho S780) antibody [EPR17624] |
| **Description** | Rabbit monoclonal [EPR17624] to Rb (phospho S780) |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** WB, IP, Dot blot |
| **Species reactivity** | **Reacts with:** Mouse, Human |
| **Immunogen** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | WB: K562 whole cell lysates untreated and serum starved for 2 days; L-929 whole cell lysate. IP: K562 whole cell lysate. |
| **General notes** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. |

## Properties

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| **Form** | Liquid |
| **Storage instructions** | 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. |
| **Storage buffer** | pH: 7.2<br>Preservative: 0.01% Sodium azide<br>Constituents: 59% PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine) |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR17624 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab184702 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| **WB** | | 1/1000. Detects a band of approximately 106 kDa (predicted molecular weight: 106 kDa). |
| **IP** | | 1/30. |
| **Dot blot** | | Use at an assay dependent concentration. |

## Target

**Function** 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.

**Tissue specificity** Expressed in the retina.

**Involvement in disease** Childhood cancer retinoblastoma
Bladder cancer
Osteogenic sarcoma

**Sequence similarities** Belongs to the retinoblastoma protein (RB) family.

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

**Post-translational modifications** 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

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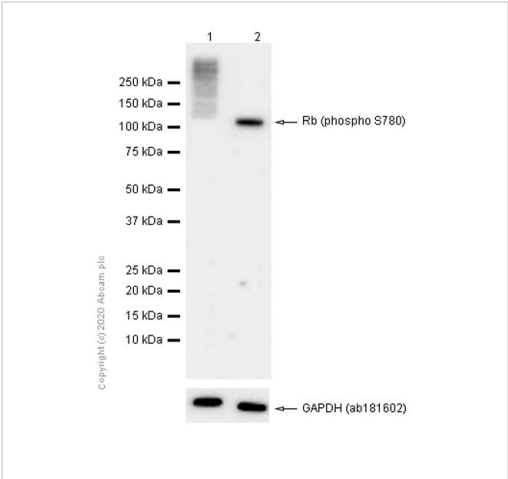
| | differentiation. |
| **Cellular localization** | Nucleus. |

## Images




Western blot - Anti-Rb (phospho S780) antibody [EPR17624] (ab184702)

**All lanes :** Anti-Rb (phospho S780) antibody [EPR17624] (ab184702) at 1/5000 dilution

**Lane 1 :** K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates prepared in RIPA lysis method
**Lane 2 :** K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates prepared in 1%SDS Hot lysis method

Lysates/proteins at 15 µg per lane.

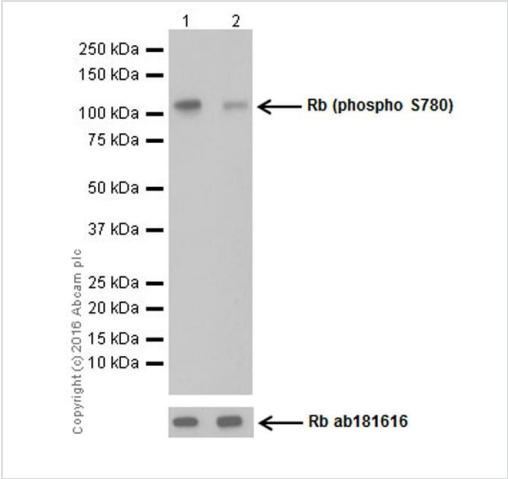
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/20000 dilution

**Predicted band size:** 106 kDa

The lysates were prepared in 1%SDS Hot lysis method

Observed MW: 106 kDa

blocking/diluting buffer and concentration: 5% NFDM/TBST




Western blot - Anti-Rb (phospho S780) antibody [EPR17624] (ab184702)

**All lanes :** Anti-Rb (phospho S780) antibody [EPR17624] (ab184702) at 1/1000 dilution

**Lane 1 :** Untreated K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate
**Lane 2 :** K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate serum starved for 2 days

Lysates/proteins at 10 µg per lane.

**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 106 kDa

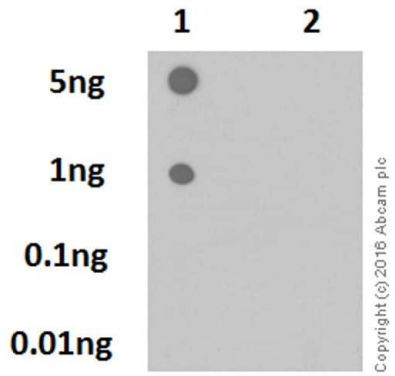
**Observed band size:** 106 kDa

**Exposure time:** 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Rb phosphorylation at S780 is regulated by cell cycle progression, which is consistent with what has been described in the literature (PMID: 11134518 and 24765199).

The lysates were prepared in 1%SDS Hot lysis method




Dot Blot - Anti-Rb (phospho S780) antibody [EPR17624] (ab184702)

Dot blot analysis of Rb (phospho S780) labeled with ab184702 at 1/1000 dilution.

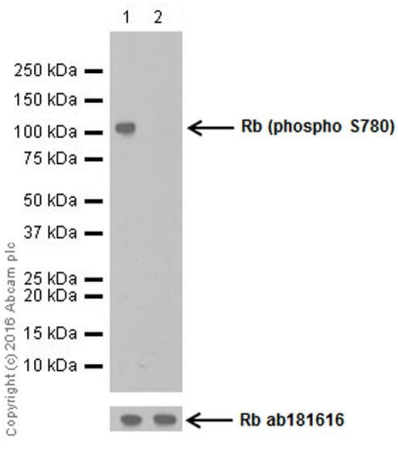
Lane 1: Rb (phospho S780) phospho peptide;

Lane 2: Rb non-phospho peptide.

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 was used as secondary antibody.

Blocking and diluting buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.




Western blot - Anti-Rb (phospho S780) antibody [EPR17624] (ab184702)

**All lanes :** Anti-Rb (phospho S780) antibody [EPR17624] (ab184702) at 1/1000 dilution

**Lane 1 :** K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate
**Lane 2 :** K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate treated with Alkaline Phosphatase for 1 hour

Lysates/proteins at 10 µg per lane.

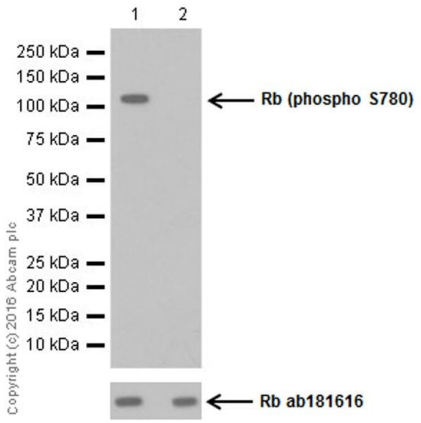
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 106 kDa
**Observed band size:** 106 kDa

**Exposure time:** 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The lysates were prepared in 1%SDS Hot lysis method




Western blot - Anti-Rb (phospho S780) antibody [EPR17624] (ab184702)

**All lanes :** Anti-Rb (phospho S780) antibody [EPR17624] (ab184702) at 1/5000 dilution

**Lane 1 :** L-929 (Mouse connective tissue fibroblast cell line) whole cell lysate
**Lane 2 :** L-929 (Mouse connective tissue fibroblast cell line) whole cell lysate treated with Alkaline Phosphatase for 1 hour

Lysates/proteins at 10 µg per lane.

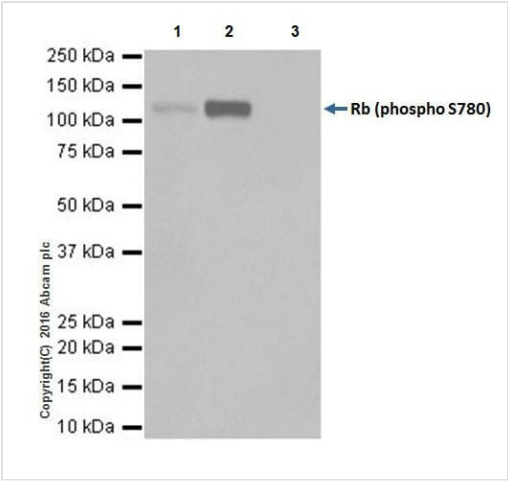
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 106 kDa
**Observed band size:** 106 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The lysates were prepared in 1%SDS Hot lysis method




Immunoprecipitation - Anti-Rb (phospho S780) antibody [EPR17624] (ab184702)

Rb (phospho S780) was immunoprecipitated from 0.35 mg of K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate with ab184702 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab184702 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

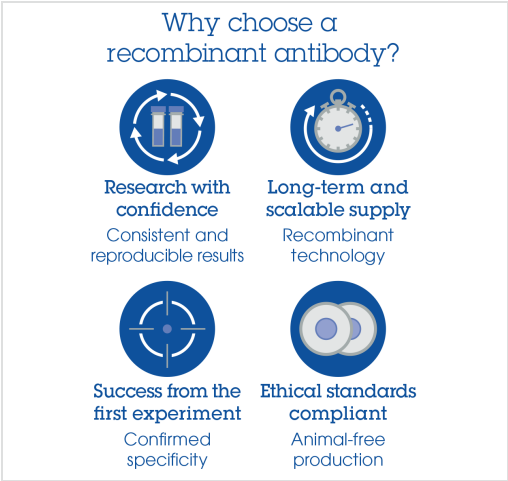
Lane 1: K562 whole cell lysate,10µg (Input).

Lane 2: ab184702 IP in K562 whole cell lysate.

Lane 3: Rabbit IgG,monoclonal [EPR25A]-Isotype Control (**ab172730**) instead of ab184702 in K562 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.




Anti-Rb (phospho S780) antibody [EPR17624] (ab184702)

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