# abcam

## Product datasheet

## Anti-RICTOR antibody [EPR22008] ab219950



Recombinant RabMAb

## 6 Images

#### Overview

**Product name** Anti-RICTOR antibody [EPR22008]

**Description** Rabbit monoclonal [EPR22008] to RICTOR

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), WB

Species reactivity Reacts with: Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HAP1, HeLa, HEK-293, HCT 116, MDA-MB-231, SW480 and PC-3 whole cell lysate. Flow

Cyt (intra): HCT 116 and HeLa cells.

**General notes** 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**.

#### **Properties**

**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

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal EPR22008 Clone number

Isotype ΙgG

### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab219950 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
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 200 kDa (predicted molecular weight: 192 kDa).

#### **Target**

#### **Function**

Subunit of mTORC2, which regulates cell growth and survival in response to hormonal signals. mTORC2 is activated by growth factors, but, in contrast to mTORC1, seems to be nutrient-insensitive. mTORC2 seems to function upstream of Rho GTPases to regulate the actin cytoskeleton, probably by activating one or more Rho-type guanine nucleotide exchange factors. mTORC2 promotes the serum-induced formation of stress-fibers or F-actin. mTORC2 plays a critical role in AKT1 'Ser-473' phosphorylation, which may facilitate the phosphorylation of the activation loop of AKT1 on 'Thr-308' by PDK1 which is a prerequisite for full activation. mTORC2 regulates the phosphorylation of SGK1 at 'Ser-422'. mTORC2 also modulates the phosphorylation of PRKCA on 'Ser-657'. Plays an essential role in embryonic growth and development.

#### Sequence similarities

Belongs to the RICTOR family.

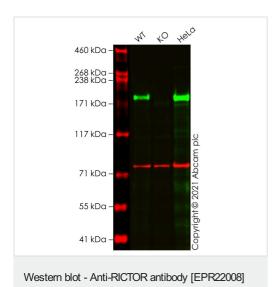
# Post-translational modifications

Phosphorylated by MTOR; when part of mTORC2. Phosphorylated at Thr-1135 by RPS6KB1;

phosphorylation of RICTOR inhibits mTORC2 and AKT1 signaling.

#### **Images**

(ab219950)



All lanes: Anti-RICTOR antibody [EPR22008] (ab219950) at

1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: RICTOR knockout A549 cell lysate

Lane 3: HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 192 kDa **Observed band size:** 190 kDa

False colour image of Western blot: Anti-RICTOR antibody [EPR22008] staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab219950 was shown to bind specifically to RICTOR. A band was observed at 190 kDa in wild-type A549 cell lysates with no signal observed at this size in RICTOR knockout cell line ab277866 (knockout cell lysate ab288315). To generate this image, wild-type and RICTOR knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-RICTOR antibody [EPR22008] (ab219950)

**All lanes :** Anti-RICTOR antibody [EPR22008] (ab219950) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate at 20 µg

Lane 2: RICTOR knockout HAP1 whole cell lysate at 20 µg

Lane 3: HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 10 µg

Lane 4: HEK-293 (human epithelial cell line from embryonic

kidney) whole cell lysate at 20 µg

## Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 192 kDa

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

Exposure time: 26 seconds.

ab219950 was shown to specifically react with RICTOR in wild-type HAP1 cells as signal was lost in RICTOR knockout cells. Wild-type and RICTOR knockout samples were subjected to SDS-PAGE. ab219950 and ab181602 (Rabbit anti-GAPDH loading control)

were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) secondary antibody at 1/50,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD<sup>®</sup> ChemiDoc™ MP instrument using the ECL technique.

2 250 kDa -250 kDa -250 kDa -250 kDa - \_ 150 kDa -150 kDa -150 kDa -150 kDa -100 kDa -100 kDa -100 kDa -100 kDa -75 kDa -75 kDa -75 kDa -75 kDa -50 kDa -50 kDa -50 kDa -50 kDa -37 kDa -37 kDa -37 kDa -37 kDa -25 kDa -(c) 2018 20 kDa -25 kDa -25 kDa -25 kDa -15 kDa -20 kDa -20 kDa -20 kDa -15 kDa -15 kDa -15 kDa -10 kDa -10 kDa -10 kDa 🕳 10 kDa -

Western blot - Anti-RICTOR antibody [EPR22008] (ab219950)

**All lanes :** Anti-RICTOR antibody [EPR22008] (ab219950) at 1/1000 dilution

**Lane 1 :** HCT 116 (Human colorectal carcinoma cell line) whole cell lysate

**Lane 2 :** MDA-MB-231 (Human breast adenocarcinoma cell line) whole cell lysate

**Lane 3 :** SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate

**Lane 4 :** PC-3 (Human prostate adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

#### **Secondary**

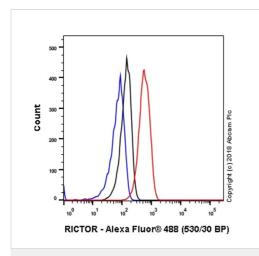
Lane 1 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Lanes 2-4: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 192 kDa

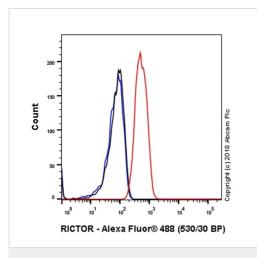
Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

Exposure times: Lane 1: 3 minutes; Lane 2: 48 seconds; Lane 3-4: 26 seconds.



Flow Cytometry (Intracellular) - Anti-RICTOR antibody [EPR22008] (ab219950)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling RICTOR with ab219950 at 1/500 (red) compared with a Rabbit monoclonal IgG (ab172730) (black) and an unlabeled control (cells incubated with secondary antibody only) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-RICTOR antibody [EPR22008] (ab219950)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HCT 116 (human colorectal carcinoma cell line) cell line labeling RICTOR with ab219950 at 1/500 (red) compared with a Rabbit monoclonal IgG (ab172730) (black) and an unlabeled control (cells incubated with secondary antibody only) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody.



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