Anti-RICTOR Antibody [1A5]

HA721142

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 192 kDa
Clone number:	1A5

Description: Rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR) is a protein that in humans is encoded by the RICTOR gene. RICTOR and mTOR are components of a protein complex that integrates nutrient- and growth factor-derived signals to regulate cell growth. RICTOR is a subunit of the mammalian target of rapamycin complex 2 (mTORC2) which contains mTOR, GβL, RICTOR (this protein) and mSIN1. The mammalian target of rapamycin (mTOR) is a highly conserved Ser/Thr kinase that regulates cell growth and proliferation. mTOR may exist as mTOR complex 1 (mTORC1) or mTOR complex 2 (mTORC2). RICTOR is a key component of mTORC2, which, unlike mTORC1, is not directly inhibited by rapamycin. mTORC2, and RICTOR, specifically, has been shown to phosphorylate Akt/protein kinase B (PKB) on SER473. This phosphorylation activates Akt/PKB, where deregulation of Akt/PKB has been implicated in cancer and diabetes. RICTOR and mTORC2 have been shown to play an essential role in embryonic growth and development, perhaps due to the control that mTORC2 exerts on actin cytoskeleton organization. Diseases associated with mutation in the RICTOR gene include foramen magnum meningioma and syringomyelia. Akt/PMB activation is also involved in glucose metabolism and activation of Akt by RICTOR has been shown to mediate glucose and lipid metabolism. Therefore, the influence of RICTOR and mTORC2 on Akt signaling has been associated with insulin resistance and type 2 diabetes.

Immunogen:	Recombinant protein within human RICTOR aa 1459-1708 / 1708.
Positive control:	HepG2 cell lysates, mouse liver tissue, A431, Hela.
Subcellular location:	Cytosol, Golgi apparatus.
Database links:	SwissProt: Q6R327 Human | Q6QI06 Mouse

Recommended Dilutions:

WB	1:500
IHC-P	1:500
IF-Cell	1:100
FC	1:500-1:1,000

Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

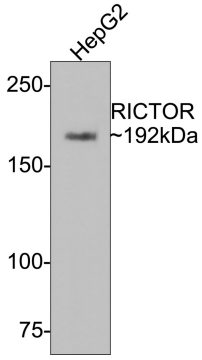

Fig1: Western blot analysis of RICTOR on HepG2 cell lysates with Rabbit anti-RICTOR antibody (HA721142) at 1/500 dilution.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 192 kDa
Observed band size: 192 kDa

Exposure time: 2 minutes;

6% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721142) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

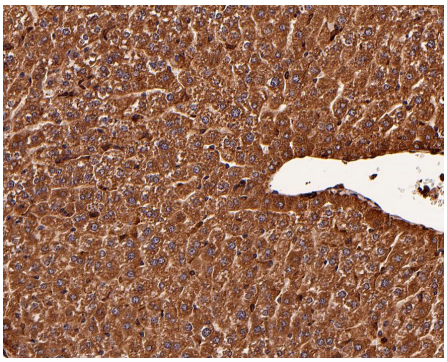

Fig2: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-RICTOR antibody (HA721142) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721142) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

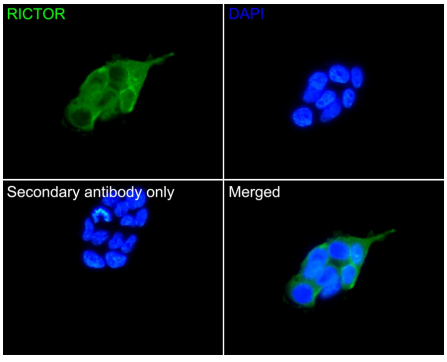

Fig3: Immunocytochemistry analysis of A431 cells labeling RICTOR with Rabbit anti-RICTOR antibody (HA721142) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-RICTOR antibody (HA721142) at 1/100 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

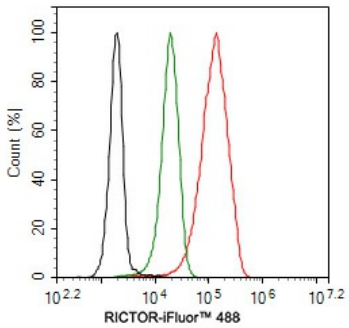

Fig4: Flow cytometric analysis of Hela cells labeling RICTOR.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721142, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Ramaiah MJ. et. al. mTOR-Rictor-EGFR axis in oncogenesis and diagnosis of glioblastoma multiforme. Mol Biol Rep. 2021 May
2. Xu S. et. al. Rictor Is a Novel Regulator of TRAF6/TRAF3 in Osteoclasts. J Bone Miner Res. 2021 Oct