abcam

Product datasheet

Anti-mTOR antibody ab45989

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Overview

Product name	Anti-mTOR antibody
Description	Rabbit polyclonal to mTOR
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-P, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 2150 - 2250 of Human mTOR.Read Abcam's proprietary immunogen policy(Peptide available as ab45988.)
Positive control	WB: HeLa, Jurkat, A431, HEK293 and HepG2 (data not shown for HepG2) whole cell lysates. IHC-P: Human testis tissue. ICC/IF: HeLa cells.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab45989 in the following tested applications.

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

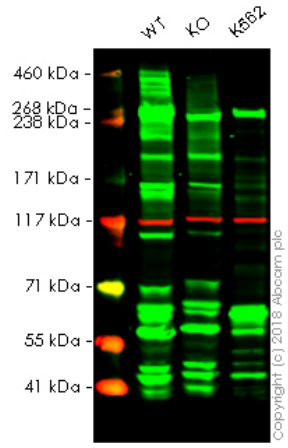
Application	Abreviews	Notes
ICC/IF		Use a concentration of 5 µg/ml.

Application	Abreviews	Notes
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	★★★★☆ (3)	Use a concentration of 1 µg/ml. Detects a band of approximately 250 kDa (predicted molecular weight: 289 kDa).

Target

Function	Kinase subunit of both mTORC1 and mTORC2, which regulates cell growth and survival in response to nutrient and hormonal signals. mTORC1 is activated in response to growth factors or amino-acids. Growth factor-stimulated mTORC1 activation involves AKT1-mediated phosphorylation of TSC1-TSC2, which leads to the activation of the RHEB GTPase that potently activates the protein kinase activity of mTORC1. Amino-acid-signaling to mTORC1 requires its relocalization to the lysosomes mediated by the Ragulator complex and the Rag GTPases. Activated mTORC1 up-regulates protein synthesis by phosphorylating key regulators of mRNA translation and ribosome synthesis. mTORC1 phosphorylates EIF4EBP1 and releases it from inhibiting the elongation initiation factor 4E (eiF4E). mTORC1 phosphorylates and activates S6K1 at 'Thr-421', which then promotes protein synthesis by phosphorylating PDCD4 and targeting it for degradation. Phosphorylates MAF1 leading to attenuation of its RNA polymerase III-repressive function. mTORC2 is also activated by growth. factors, but 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'.
Tissue specificity	Expressed in numerous tissues, with highest levels in testis.
Sequence similarities	Belongs to the PI3/PI4-kinase family. Contains 1 FAT domain. Contains 1 FATC domain. Contains 7 HEAT repeats. Contains 1 PI3K/PI4K domain.
Post-translational modifications	Autophosphorylated; when part of mTORC1 or mTORC2.
Cellular localization	Endoplasmic reticulum membrane. Golgi apparatus membrane. Mitochondrion outer membrane. Lysosome. Cytoplasm. Nucleus > PML body. Shuttles between cytoplasm and nucleus. Accumulates in the nucleus in response to hypoxia (By similarity). Targeting to lysosomes depends on amino acid availability and RRAGA and RRAGB.

Images



Western blot - Anti-mTOR antibody (ab45989)

All lanes : Anti-mTOR antibody (ab45989) at 1 µg/ml

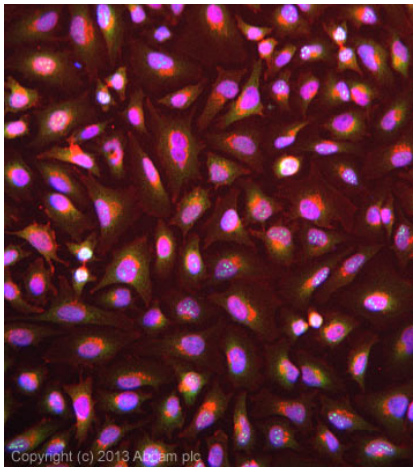
Lane 1 : Wild-type HEK293T whole cell lysate
Lane 2 : MTOR knockout HEK293T whole cell lysate
Lane 3 : K562 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 289 kDa

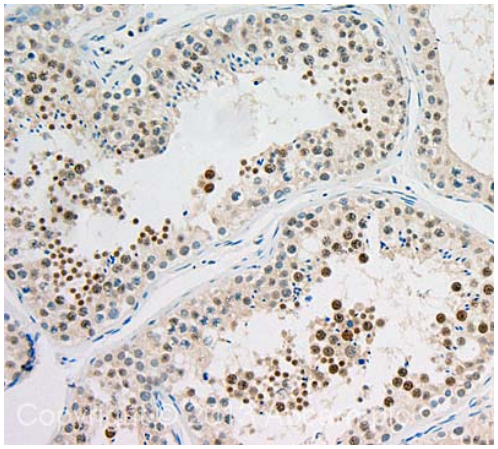
Lanes 1 - 3: Merged signal (red and green). Green - ab45989 observed at 289 kDa. Red - loading control, ab130007, observed at 130 kDa.

ab45989 was found to be non-specific when MTOR knockout samples were used. Wild-type and MTOR knockout samples were subjected to SDS-PAGE. Ab45989 and ab130007 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 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 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-mTOR antibody (ab45989)

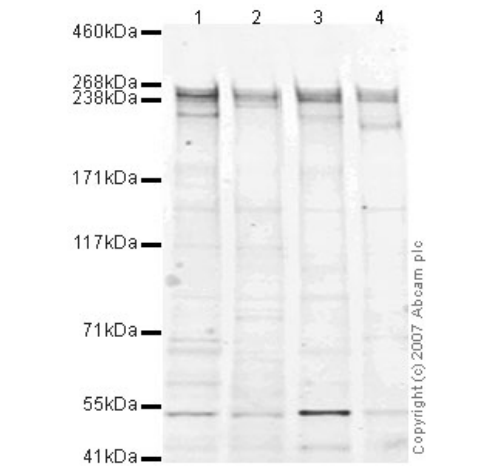
ICC/IF image of ab45989 stained HeLa cells. The cells were 4% Formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal Goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab45989, 5µg/ml) overnight at +4°C. The secondary antibody (green) was ab96899, DyLight® 488 Goat anti-Rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR antibody (ab45989)

IHC image of mTOR staining in Human Testis formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab45989, 1 µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-mTOR antibody (ab45989)

All lanes : Anti-mTOR antibody (ab45989) at 1 µg/ml

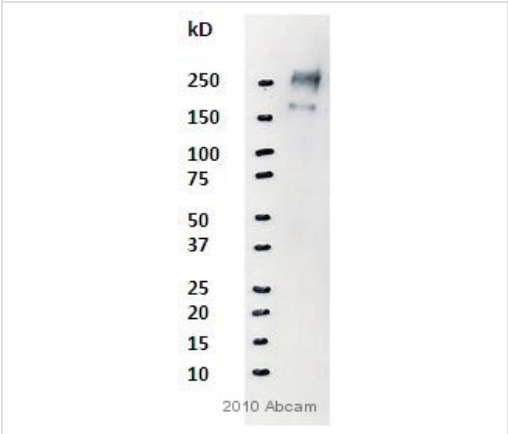
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
Lane 3 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 4 : HEK293 Human embryonic kidney cell line Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 289 kDa
Observed band size: 250 kDa



Western blot - Anti-mTOR antibody (ab45989)

This image is courtesy of an anonymous Abreview

Anti-mTOR antibody (ab45989) at 1 µg/ml + Human HEK293 whole cell lysate at 12.5 µg

Secondary

An HRP-conjugated Goat anti-rabbit IgG polyclonal at 1/16000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 289 kDa
Observed band size: 289 kDa
Additional bands at: 180 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 5 minutes

Blocking Step: 5% Milk for 45 minutes at room temperature.

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