


Product datasheet

Anti-mTOR antibody ab2732

★★★★☆ [14 Abreviews](#) [331 References](#) [5 Images](#)

Overview

Product name	Anti-mTOR antibody
Description	Rabbit polyclonal to mTOR
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, IP, WB
Species reactivity	Reacts with: Rat, Human Predicted to work with: Mouse 
Immunogen	Synthetic peptide within Human mTOR aa 200-250. The exact sequence is proprietary. Database link: P42345 (Peptide available as ab39393)
Positive control	ICC/IF: HepG2 cells. L6 myotubes. IP: HeLa lysates. WB: HeLa, HEK-293T, Jurkat-HepG2 and LNCaP whole cell lysate.
General notes	<p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7 Preservative: 0.1% Sodium azide Constituents: 0.021% PBS, 1.764% Sodium citrate, 1.815% Tris
Purification notes	Affinity purified using the immunising peptide immobilized on solid support.
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab2732 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	★★★★★ (1)	1/100.
IP		Use at 2-10 µg/mg of lysate.
WB	★★★★★ (7)	1/2000 - 1/10000. 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 potentially 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.

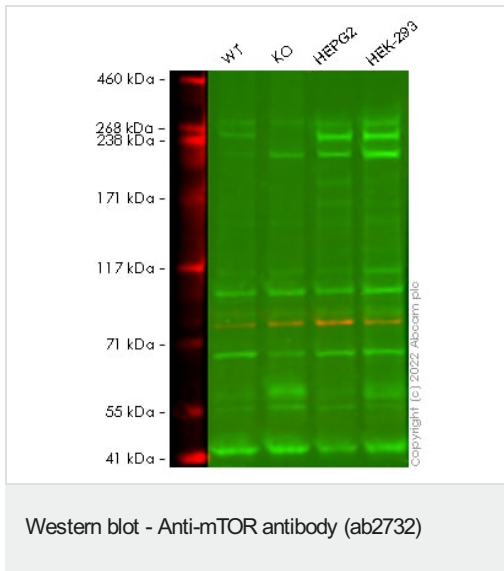
Post-translational modifications

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



All lanes : Anti-mTOR antibody (ab2732) at 1/2000 dilution

Lane 1 : Wild-type A549 cell lysate

Lane 2 : MTOR [homo] CRISPR-Cas9 edited A549 cell lysate

Lane 3 : HepG2 cell lysate

Lane 4 : HEK-293 cell lysate

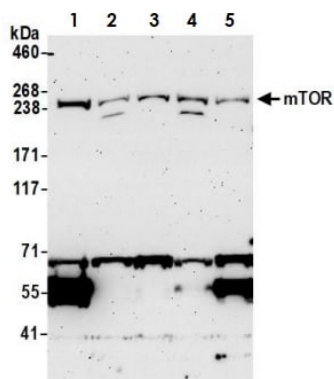
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 289 kDa

Observed band size: 250 kDa

False colour image of Western blot: Anti-mTOR antibody staining at 1/2000 dilution, shown in green; Mouse anti-CANX [CANX/1543] ([ab238078](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab2732 was shown to bind specifically to mTOR. A band was observed at 250 kDa in wild-type A549 cell lysates with no signal observed at this size in MTOR CRISPR-Cas9 edited cell line [ab283257](#). The band observed in the CRISPR-Cas9 edited lysate lane below 250 kDa is likely to represent a truncated form of mTOR. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and MTOR CRISPR-Cas9 edited 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-mTOR antibody (ab2732)

All lanes : Anti-mTOR antibody (ab2732) at 0.1 µg/ml

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 3 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 4 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

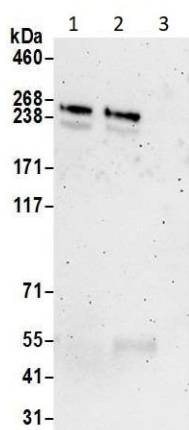
Lane 5 : LNCaP (Human prostate cancer cell line) whole cell lysate

Lysates/proteins at 50 µg per lane.

Predicted band size: 289 kDa

Exposure time: 3 minutes

Lysates prepared using NETN lysis buffer.



Immunoprecipitation - Anti-mTOR antibody (ab2732)

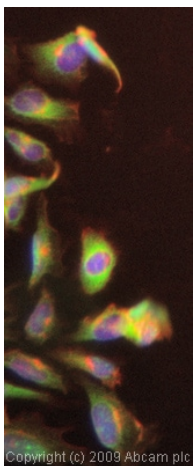
mTOR was immunoprecipitated from HeLa cell lysate (1.0 mg per IP reaction; 20% of IP loaded) with ab2732 at 3 µg per reaction. mTOR was also immunoprecipitated by **ab2833**. Western blot was performed from the immunoprecipitate with ab2732 at 1 µg/ml.

Lane 1: **ab2833** IP in HeLa whole cell lysate.

Lane 2: ab2732 IP in HeLa whole cell lysate.

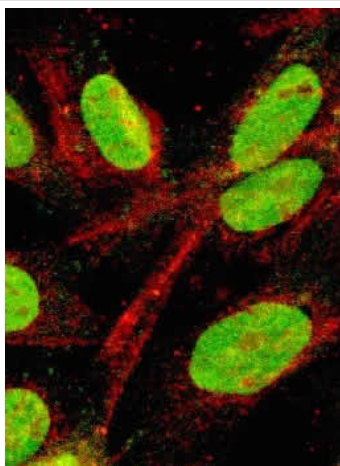
Lane 3: Control IgG IP in HeLa whole cell lysate.

Detection: Chemiluminescence



Immunocytochemistry/ Immunofluorescence - Anti-mTOR antibody (ab2732)

ICC/IF image of ab2732 stained HepG2 cells. The cells were 100% methanol fixed (5 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 (ab2732, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 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.



Immunocytochemistry/ Immunofluorescence - Anti-mTOR antibody (ab2732)

ab2732 at a 1:100 dilution confocally staining mTOR (red) in L6 myotubes, alongside a nuclear antigen antibody (green).

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