

Product datasheet

Anti-mTOR antibody ab2732

★★★★☆ 10 Abreviews 48 References 6 Images

Overview

Product name	Anti-mTOR antibody
Description	Rabbit polyclonal to mTOR
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, WB, IHC-Fr, IP, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human, Equus Predicted to work with: Sheep, Rabbit, Goat, Guinea pig, Cow, Dog, Pig, Ferret, Rhesus monkey, Elephant
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. IHC-P: Rat and mouse testis tissue. IP: HEK-293T lysates.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	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

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		Use at an assay dependent concentration.

Application	Abreviews	Notes
WB	★★★★☆	1/2000.
IHC-Fr		1/100.
IP		1/1000.
IHC-P	★★★★☆	1/2000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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.

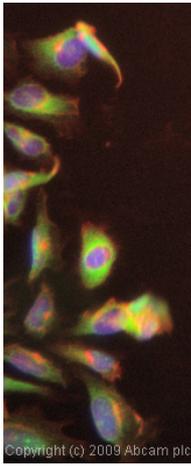
Post-translational modifications

Autophosphorylated; when part of mTORC1 or mTORC2.

Cellular localization

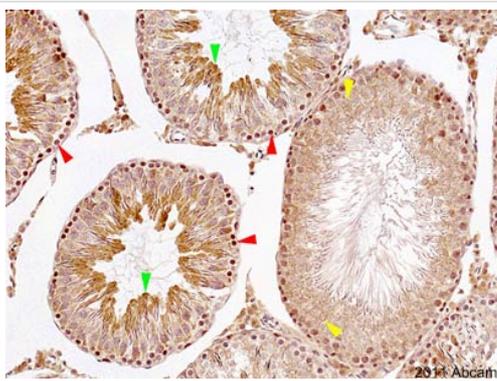
Endoplasmic reticulum membrane. Golgi apparatus membrane. Mitochondrion outer membrane. Lysosome. Cytoplasm. Nucleus > PML body. Shuttles between cytoplasm and nucleus. Accumulates in the nucleus in response to hypoxia (By similarity). Targeting to lysosomes depends on amino acid availability and RRAGA and RRAGB.

Images



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.

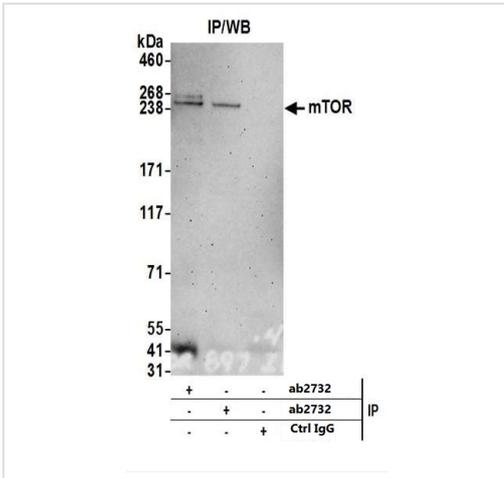


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR antibody (ab2732)

This image is courtesy of an Abreview submitted by Carl Hobbs

ab2732 staining rat testis sections by IHC-P. The tissue was fixed with formaldehyde and a heat mediated antigen retrieval step was performed with citric acid pH 6. Blocking of the sample was done with 1%BSA for 10 minutes at 21°C, followed by staining with ab2732 at 1/2000 in TBS/BSA/azide for 2h at 21°C. A biotinylated goat anti-rabbit polyclonal antibody at 1/200 was used as the secondary antibody.

Positive components in the submitted image are indicated by coloured arrowheads: red for densely positive nuclei that appear to be confined to the stem cell layer (immediately above, some spermatogonia exhibit a nuclear speckling). Yellow indicates a moderate cytoplasmic positivity, more evident in some seminiferous tubules (ST). Green for a strong apparently cytoplasmic positivity at the luminal surface of a proportion of ST.



Immunoprecipitation - Anti-mTOR antibody (ab2732)

Detection of Human mTOR by Western Blot of Immunoprecipitates.

Samples: Whole cell lysate (0.5 or 1.0 mg per IP reaction; 20% of IP loaded) from 293T cells prepared using lysis buffer.

Antibodies: Anti-mTOR antibody (ab2732) used for IP at 6 µg per reaction.

For blotting immunoprecipitated mTOR, ab2732 was used at 0.4 µg/ml.

Detection: Chemiluminescence with an exposure time of 3 minutes.

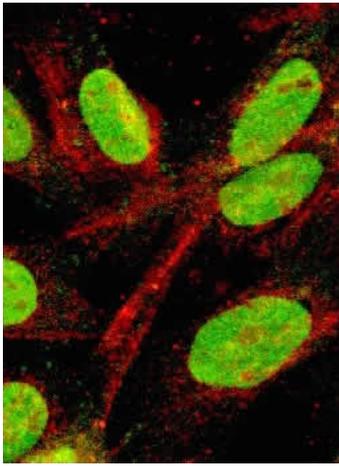


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR antibody (ab2732)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

ab2732 staining mouse testis sections by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). The tissue was fixed with formaldehyde and a heat mediated antigen retrieval step was performed with citric acid pH 6. Blocking of the sample was done with 1%BSA for 10 minutes at 21°C, followed by staining with ab2732 at 1/3000 in TBS/BSA/azide for 2h at 21°C. A biotinylated goat anti-rabbit polyclonal antibody at 1/200 was used as the secondary antibody.

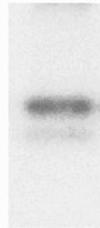
Positive components in the submitted image are indicated by coloured arrowheads: positive nuclei (red) appear to be confined to the stem cell layer of the seminiferous tubules whereas maturing cells appear to have light cytoplasmic positivity. In all tubes there is a luminal cytoplasmic positivity that varies in intensity (green) between tubules. Leydig cells are also positive (yellow). Spaces around the tubes are artefactual.



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

Immunocytochemistry/ Immunofluorescence - Anti-mTOR antibody (ab2732)

4E-BP1 →



mTOR protein kinase assay:

ab2732 (1:1000 dilution) immunoprecipitates were incubated at 30°C with recombinant 4E-BP1 and 32P-gATP. Autoradiograph shows 32P incorporated into 4E-BP1.

Immunoprecipitation - Anti-mTOR antibody (ab2732)

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