Product datasheet

Anti-mTOR (phospho S2448) antibody ab84400

Overview

Product name
Anti-mTOR (phospho S2448) antibody

Description
Rabbit polyclonal to mTOR (phospho S2448)

Host species
Rabbit

Tested applications
Suitable for: IHC-P, WB, ICC/IF

Species reactivity
Reacts with: Human
Predicted to work with: Mouse, Rat, Sheep, Rabbit, Goat, Horse, Cow, Dog, Pig, Zebrafish, Macaque monkey, Gorilla, Chinese hamster

Immunogen
Synthetic peptide conjugated to KLH derived from within residues 2400 - 2500 of Human mTOR, phosphorylated at S2448.Read Abcam's proprietary immunogen policy

Positive control
WB: HeLa, Jurkat and A431 (EGF treated) whole cell lysates. IHC-P: Human normal hippocampus tissue. ICC/IF: methanol fixed MCFM 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
pH: 7.40
Preservative: 0.02% Sodium azide
Constituent: PBS

Note: Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity
Immunogen affinity purified

Clonality
Polyclonal

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab84400 in the following tested applications.

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


**Application**

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
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<td></td>
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</tbody>
</table>

**Notes**

IHC-P
Use a concentration of 1 µg/ml.
WB
Use a concentration of 1 µg/ml. Detects a band of approximately 268 kDa (predicted molecular weight: 288 kDa).
ICC/IF
Use a concentration of 5 µg/ml.
All lanes: Anti-mTOR (phospho S2448) antibody (ab84400) at 1 µg/ml

Lane 1: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2: EGF-Stimulated A431 Whole Cell Lysate
Lane 3: Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
Lane 4: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Immunizing peptide at 1 µg/ml
Lane 5: EGF-Stimulated A431 Whole Cell Lysate with Immunizing peptide at 1 µg/ml
Lane 6: Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate with Immunizing peptide at 1 µg/ml

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 288 kDa
Observed band size: 268 kDa
Additional bands at: 117 kDa, 41 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 minutes
IHC image of mTOR staining in Human hippocampus formalin fixed paraffin embedded tissue section, performed on a Leica BondTM 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 ab84400, 1µg/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.

ICC/IF image of ab84400 stained MCF-7 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 ab84400 at 5µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) 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.

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

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