


## Product datasheet

# Anti-mTOR (phospho S2448) antibody [EPR426(2)] ab109268

Recombinant RabMAb

★★★★★ 6 Abreviews 441 References 10 Images

### Overview

Product name	Anti-mTOR (phospho S2448) antibody [EPR426(2)]
Description	Rabbit monoclonal [EPR426(2)] to mTOR (phospho S2448)
Host species	Rabbit
Tested applications	<b>Suitable for:</b> WB, IHC-P, Dot blot, IHC-Fr <b>Unsuitable for:</b> Flow Cyt, ICC/IF or IP
Species reactivity	<b>Reacts with:</b> Mouse, Human <b>Predicted to work with:</b> Rat, Pig 
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK293, HeLa and NIH/3T3 cell lysates. IHC-P: Human breast and endometrium carcinoma tissue. Dot Blot: mTOR (phospho S2448) phospho peptide. IHC-Fr: Human heart tissue sections.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

### 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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
Purity	Protein A purified

<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR426(2)
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab109268 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
WB	★★★★★ (4)	1/1000 - 1/10000. Predicted molecular weight: 289 kDa.
IHC-P	★★★★★ (1)	1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Dot blot		1/1000.
IHC-Fr		Use at an assay dependent concentration.

**Application notes** Is unsuitable for Flow Cyt, ICC/IF or IP.

## Target

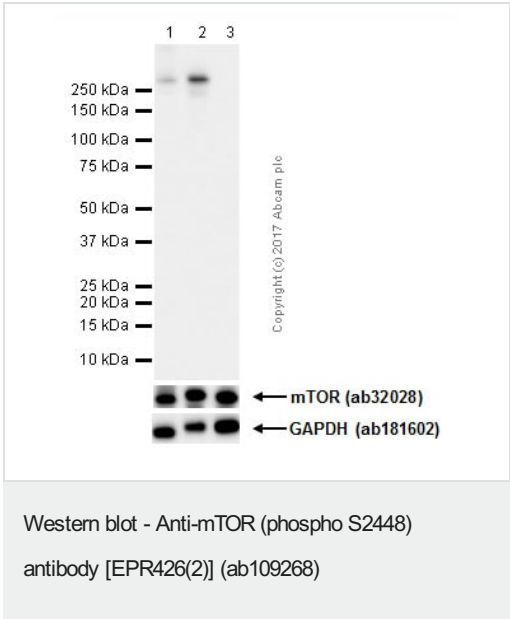
**Function** Kinase subunit of both mTORC1 and mTORC2, which regulates cell growth and survival in response to nutrient and hormonal signals. mTORC1 is activated in response to growth factors or amino-acids. Growth factor-stimulated mTORC1 activation involves AKT1-mediated phosphorylation of TSC1-TSC2, 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 (phospho S2448) antibody [EPR426(2)] (ab109268) at 1/2000 dilution (Purified)

**Lane 1 :** HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

**Lane 2 :** HeLa grown in serum-free media overnight, then treated with 200nM PMA for 4 hours whole cell lysate

**Lane 3 :** HeLa grown in serum-free media overnight, then treated with 200nM PMA for 4 hours whole cell lysate. Then the membrane was incubated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

Secondary

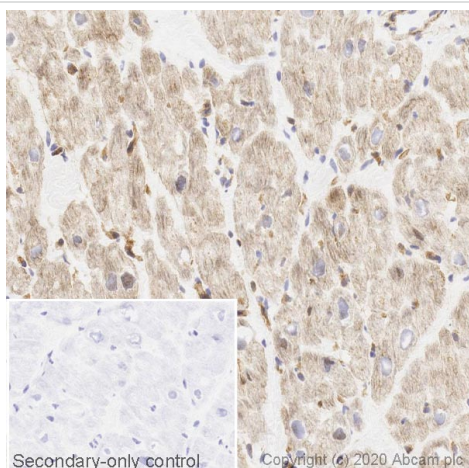
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

**Predicted band size:** 289 kDa

**Observed band size:** 289 kDa

Blocking buffer: 5% NFDM/TBST

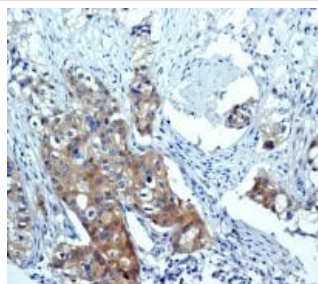
Diluting buffer: 5% NFDM /TBST



Immunohistochemistry (Frozen sections) - Anti-mTOR (phospho S2448) antibody [EPR426(2)] (ab109268)

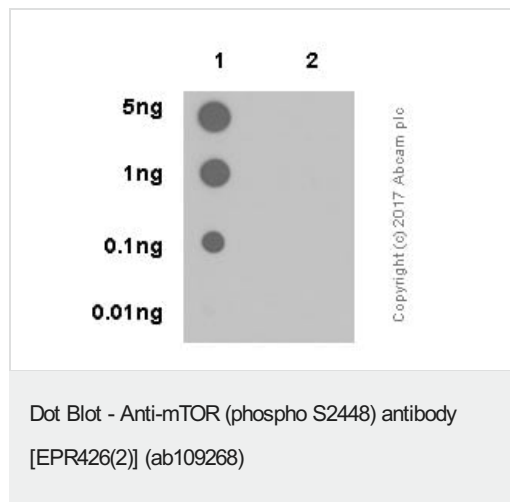
IHC image of mTOR (phospho S2448) staining in a section of frozen normal human heart performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab109268, 1/200 dilution, for 15 mins at room temperature 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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR (phospho S2448) antibody [EPR426(2)] (ab109268)

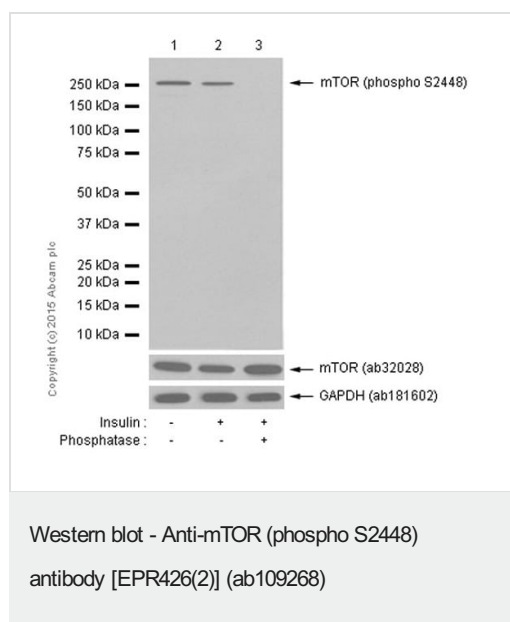
Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using unpurified ab109268 at a dilution of 1/50. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Dot blot analysis of mTOR (phospho S2448) phospho peptide (Lane 1) and mTOR non-phospho peptide (Lane 2) labeling mTOR (phospho S2448) phospho peptide with purified ab109268 at a dilution of 1/1000 (0.073ug/ml). A Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) was used as the secondary antibody at a dilution of 1/100,000.

Blocking buffer: 5% NFDM/TBST

Diluting buffer: 5% NFDM /TBST



**All lanes :** Anti-mTOR (phospho S2448) antibody [EPR426(2)] (ab109268) at 1/1000 dilution (purified)

**Lane 1 :** untreated NIH/3T3 cell lysate

**Lane 2 :** NIH/3T3 cell lysate treated with insulin

**Lane 3 :** NIH/3T3 cell lysate treated with insulin, then the membrane treated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (HRP goat anti-rabbit IgG (H+L))

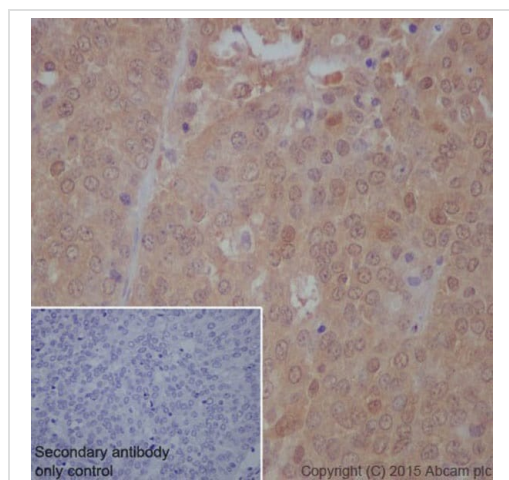
**Predicted band size:** 289 kDa

**Observed band size:** 289 kDa

**Exposure time:** 2 minutes

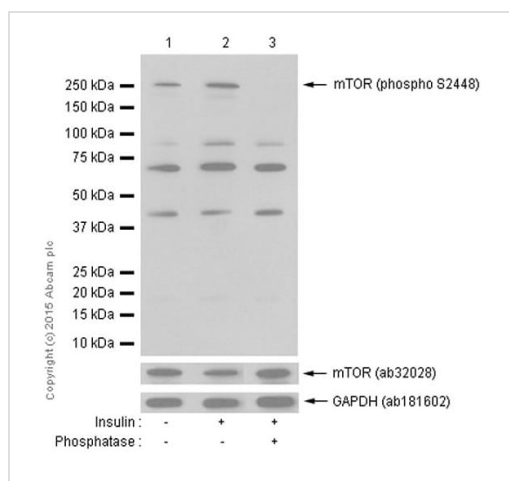
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR (phospho S2448) antibody [EPR426(2)] (ab109268)

Immunohistochemical cytoplasmic and nuclear staining of paraffin embedded human endometrium carcinoma with purified ab109268 at a working dilution of 1 in 100. The secondary antibody used is **ab97051**, a HRP goat anti-rabbit IgG (H+L), at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Western blot - Anti-mTOR (phospho S2448) antibody [EPR426(2)] (ab109268)

**All lanes :** Anti-mTOR (phospho S2448) antibody [EPR426(2)] (ab109268) at 1/1000 dilution (purified)

**Lane 1 :** untreated HeLa cell lysate

**Lane 2 :** HeLa cell lysate treated with insulin

**Lane 3 :** HeLa cell lysate treated with insulin, and the membrane treated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/2000 dilution (HRP goat anti-rabbit (H+L))

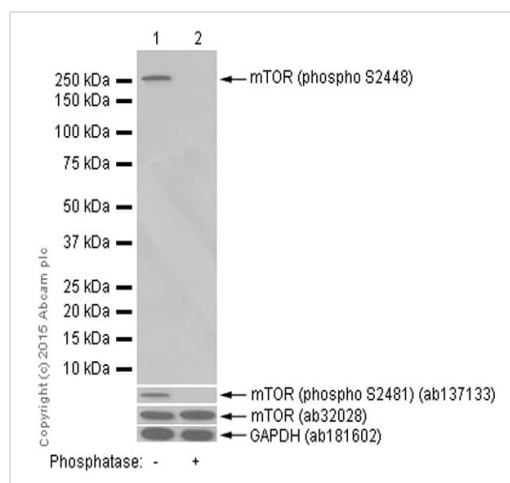
**Predicted band size:** 289 kDa

**Observed band size:** 289 kDa

**Exposure time:** 1 minute

Blocking buffer: 2% BSA/TBST

Dilution buffer: 2% BSA/TBST



Western blot - Anti-mTOR (phospho S2448)  
antibody [EPR426(2)] (ab109268)

**All lanes :** Anti-mTOR (phospho S2448) antibody [EPR426(2)]  
(ab109268) at 1/2000 dilution (purified)

**Lane 1 :** untreated HEK293 cell lysate

**Lane 2 :** HEK293 cell lysate treated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000  
dilution (HRP goat anti-rabbit IgG (H+L))

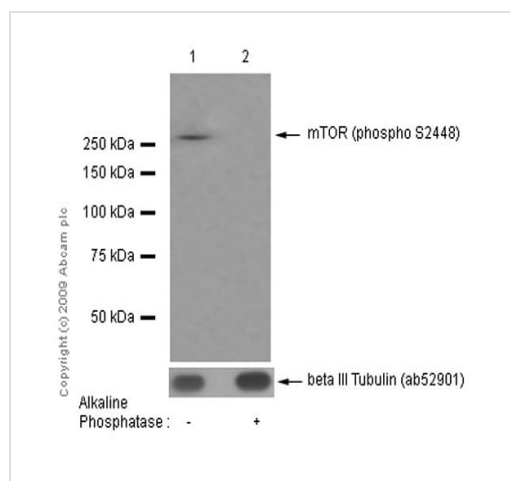
**Predicted band size:** 289 kDa

**Observed band size:** 289 kDa

**Exposure time:** 3 minutes

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-mTOR (phospho S2448)  
antibody [EPR426(2)] (ab109268)

**All lanes :** Anti-mTOR (phospho S2448) antibody [EPR426(2)]  
(ab109268) at 1/5000 dilution (unpurified)

**Lane 1 :** untreated HEK293 cell lysate

**Lane 2 :** HEK293 cell lysate treated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/2000  
dilution (HRP goat anti-rabbit IgG (H+L))

**Predicted band size:** 289 kDa

**Observed band size:** 289 kDa

**Exposure time:** 3 minutes

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

Why choose a recombinant antibody?

**Research with confidence**  
Consistent and reproducible results

**Long-term and scalable supply**  
Recombinant technology

**Success from the first experiment**  
Confirmed specificity

**Ethical standards compliant**  
Animal-free production

Anti-mTOR (phospho S2448) antibody [EPR426(2)]  
(ab109268)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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