abcam

Product datasheet

# Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free ab177734

重组 RabMAb

10 图像

## 概述

| | |
|---|---|
| **产品名称** | Anti-mTOR (phospho S2448)**抗体**[EPR426(2)] - BSA and Azide free |
| **描述** | **兔单克隆抗体**[EPR426(2)] to mTOR (phospho S2448) - BSA and Azide free |
| **宿主** | Rabbit |
| **经测试应用** | **适用于:** IHC-P, WB, IHC-Fr, Dot blot<br>**不适用于:** Flow Cyt,ICC/IF or IP |
| **种属反应性** | **与反应:** Mouse, Human<br>**预测可用于:** Rat, Pig |
| **免疫原** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **常规说明** | ab177734 is the carrier-free version of **ab109268**.<br><br>Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.<br><br>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.<br><br>Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.<br><br>This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.<br><br>This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br><br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. |

## 性能

| | |
|---|---|
| **形式** | Liquid |
| **存放说明** | Shipped at 4°C. Store at +4°C. Do Not Freeze. |
| **存储溶液** | Constituent: PBS |
| **无载体** | **是** |
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |
| **克隆编号** | EPR426(2) |
| **同种型** | IgG |

## 应用

**The Abpromise guarantee** **Abpromise™承诺保证使用ab177734于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

| 应用 | Ab评论 | 说明 |
|---|---|---|
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. |
| **WB** | | Use at an assay dependent concentration. Predicted molecular weight: 289 kDa. |
| **IHC-Fr** | | Use at an assay dependent concentration. |
| **Dot blot** | | Use at an assay dependent concentration. |

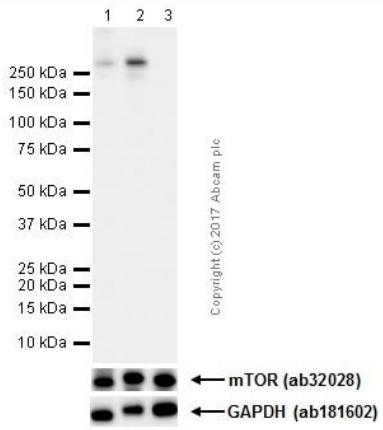
**应用说明** Is unsuitable for Flow Cyt,ICC/IF or IP.

## 靶标

**功能** 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'. |
| **组织特异性** | Expressed in numerous tissues, with highest levels in testis. |
| **序列相似性** | Belongs to the PI3/PI4-kinase family.<br>Contains 1 FAT domain.<br>Contains 1 FATC domain.<br>Contains 7 HEAT repeats.<br>Contains 1 PI3K/PI4K domain. |
| **翻译后修饰** | Autophosphorylated; when part of mTORC1 or mTORC2. |
| **细胞定位** | 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. |

## 图片




Western blot - Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

**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

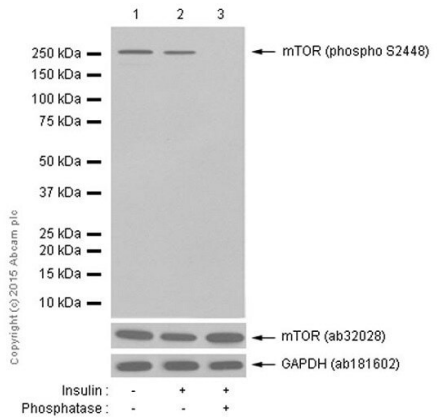
**Predicted band size:** 289 kDa
**Observed band size:** 289 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109268**).

Blocking buffer: 5% NFDM/TBST

Dilutiing buffer: 5% NFDM /TBST

**ab181602** was used as a GAPDH loading control.




Western blot - Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

**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 wth 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

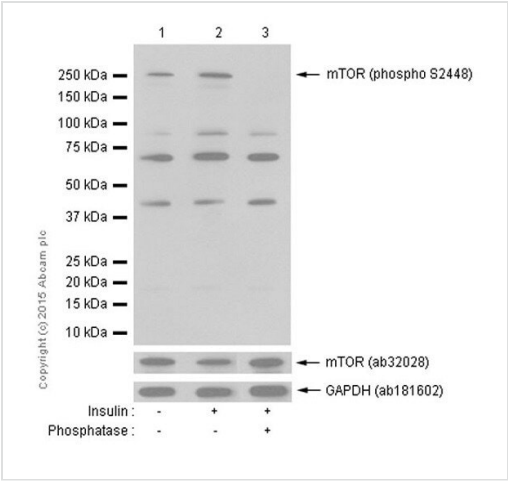
**Exposure time:** 2 minutes

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109268**).

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

**ab181602** was used as a GAPDH loading control.




Western blot - Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

**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 lystae 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

**Predicted band size:** 289 kDa
**Observed band size:** 289 kDa

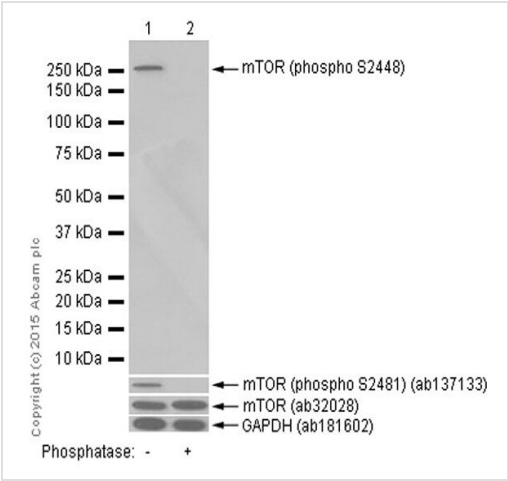
**Exposure time:** 1 minute

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109268**).

Blocking buffer: 2% BSA/TBST

Dilution buffer: 2% BSA/TBST

**ab181602** was used as a GAPDH loading control.




Western blot - Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

**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

**Predicted band size:** 289 kDa
**Observed band size:** 289 kDa

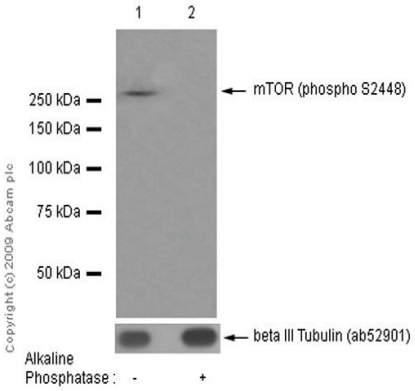
**Exposure time:** 3 minutes

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109268**).

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

**ab181602** was used as a GAPDH loading control.




Western blot - Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

**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

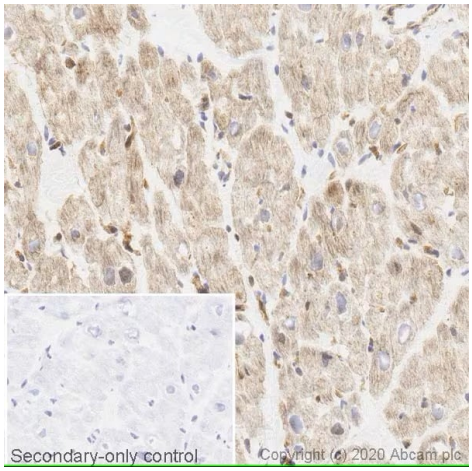
**Predicted band size:** 289 kDa
**Observed band size:** 289 kDa

**Exposure time:** 3 minutes

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109268**).

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

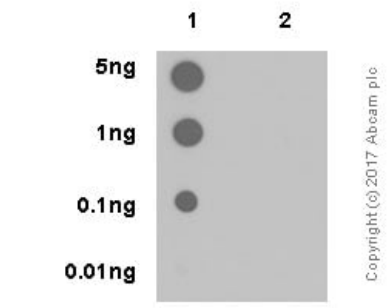



Immunohistochemistry (Frozen sections) - Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**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.

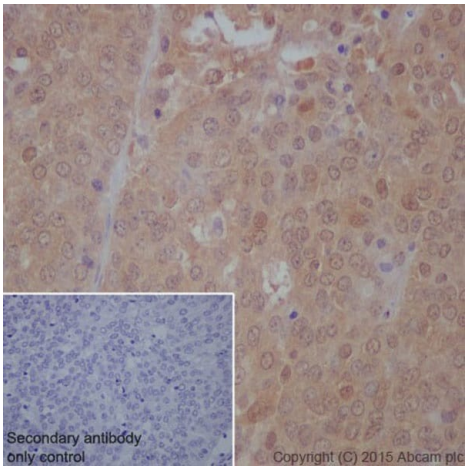



Dot Blot - Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

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

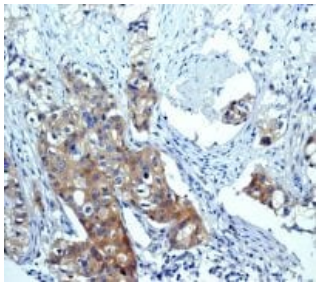
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109268**).




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

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 perfomed 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109268**).

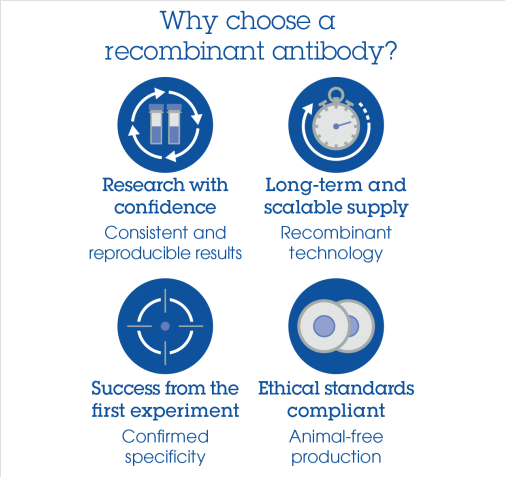


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using unpurified **ab109268** at a dilution of 1/50.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109268**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.




Anti-mTOR (phospho S2448) antibody [EPR426(2)] - BSA and Azide free (ab177734)

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

## Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.cn/abpromise** or contact our technical team.

## Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors