abcam

Product datasheet

Anti-mTOR antibody [Y391] ab32028

重组 RabMAb

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概述

产品名称	Anti-mTOR抗体[Y391]
描述	兔单克隆抗体[Y391] to mTOR
宿主	Rabbit
特异性	Expression levels of the target protein vary with sample type and some optimisation may be required.
经测试应用	**适用于:** WB, IP, IHC-P, IHC-Fr **不适用于:** Flow Cyt or ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human mTOR aa 2400-2500 (C terminal). The exact sequence is proprietary. (Peptide available as **ab193663**)
表位	ab32028 reacts with an epitope located in the C terminal region of mTOR.
阳性对照	WB: Jurkat whole cell lysate (**ab30128**), HeLa, HaCaT and MDA-MB-231 cell lysates and rat brain tissue lysate. IHC-P: Human breast carcinoma, mouse testis and rat testis tissues. IP: Rat brain tissue lysate and HeLa whole cell lysate (**ab150035**). IHC-Fr: Human heart tissue sections.
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20

Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

纯度	Protein A purified
克隆	单克隆
克隆编号	Y391
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab32028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
WB	★★★☆☆ (3)	1/1000 - 1/5000. Detects a band of approximately 250 kDa (predicted molecular weight: 289 kDa). This antibody detects non-specific bands and high background. It doesn't detect the target band in some mouse and rat tissues.
IP		1/50 - 1/100.
IHC-P	★★★★☆ (1)	1/400. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
IHC-Fr		1/200.

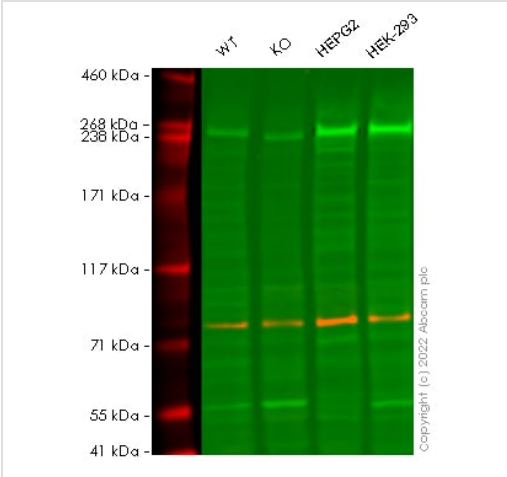
应用说明 Is unsuitable for Flow Cyt or ICC/IF.

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. Contains 1 FAT domain. Contains 1 FATC domain. Contains 7 HEAT repeats. 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 antibody [Y391] (ab32028)

All lanes : Anti-mTOR antibody [Y391] (ab32028) at 1/1000 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

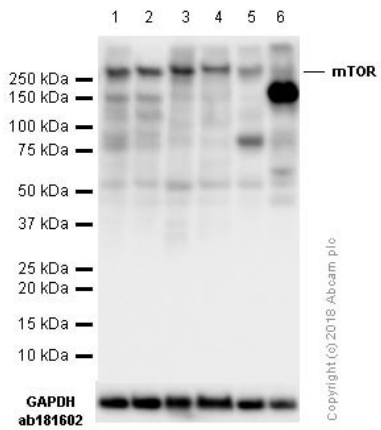
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 289 kDa
Observed band size: 250 kDa

False colour image of Western blot: Anti-mTOR antibody [Y391] staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32028 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 [Y391] (ab32028)

All lanes : Anti-mTOR antibody [Y391] (ab32028) at 1/2000 dilution

Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates in RIPA buffer
Lane 2 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates in HOT buffer
Lane 3 : Rat brain lysates in RIPA buffer
Lane 4 : Rat brain lysates in HOT buffer
Lane 5 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates
Lane 6 : Human fetal lung lysates

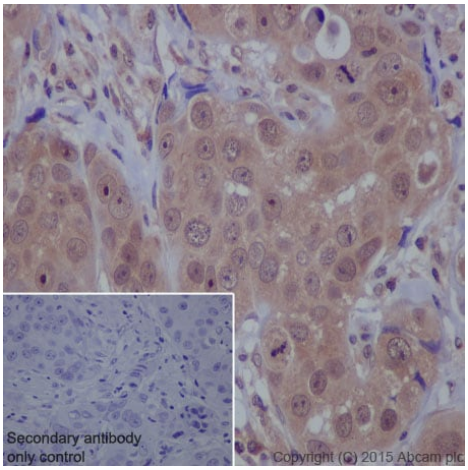
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 289 kDa
Observed band size: 290 kDa

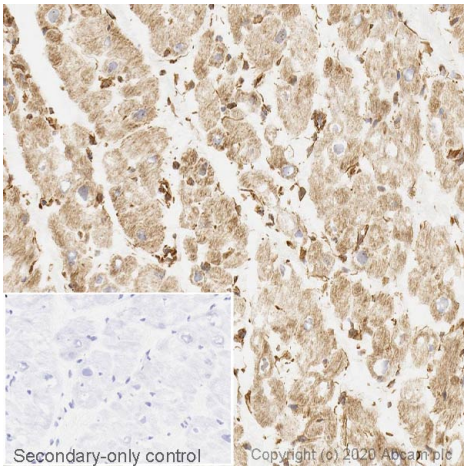
Exposure time: 15 seconds

Blocking and diluting buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR antibody [Y391] (ab32028)

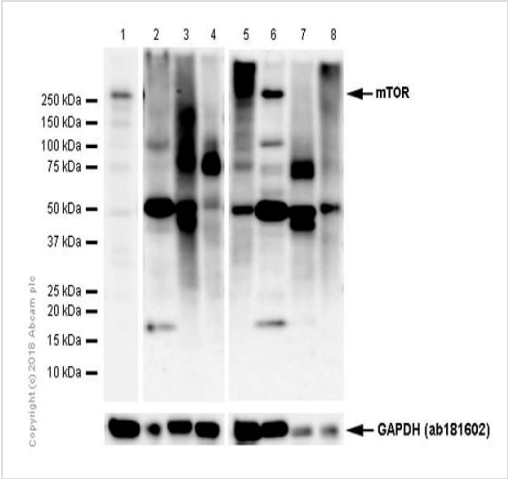
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling mTOR with purified ab32028 at a dilution of 1/400. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Frozen sections) - Anti-mTOR antibody [Y391] (ab32028)

IHC image of mTOR 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 ab32028, 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.



Western blot - Anti-mTOR antibody [Y391] (ab32028)

Lanes 1-7 : Anti-mTOR antibody [Y391] (ab32028)
Lane 8 : Anti-mTOR antibody [Y391] (ab32028) at 1/1000 dilution

Lane 1 : Rat brain tissue lysate with 5% NFDM/TBST
Lane 2 : Rat heart tissue lysate with 5% NFDM/TBST
Lane 3 : Rat liver tissue lysate with 5% NFDM/TBST
Lane 4 : Rat spleen tissue with 5% NFDM/TBST
Lane 5 : Mouse brain tissue lysate with 5% NFDM/TBST
Lane 6 : Mouse heart tissue lysate with 5% NFDM/TBST
Lane 7 : Mouse kidney tissue lysate with 5% NFDM/TBST
Lane 8 : Mouse liver tissue lysate with 5% NFDM /TBST

Lysates/proteins at 20 µg per lane.

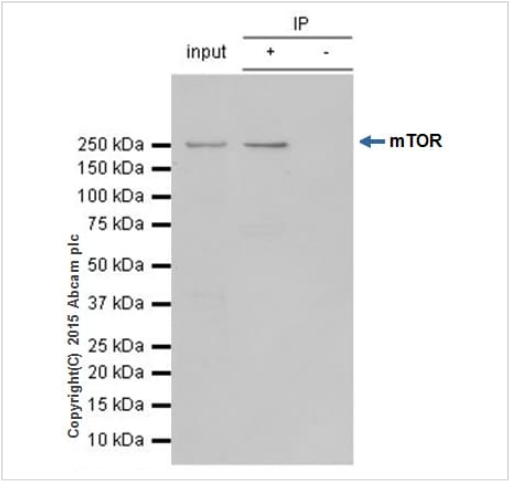
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

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

Exposure time:

Lane 1: 10 seconds
Lane 2-8: 180 seconds

This antibody detects non-specific bands and high background. It doesn't detect the target band in some mouse and rat tissues.



Immunoprecipitation - Anti-mTOR antibody [Y391] (ab32028)

ab32028 (purified) at a dilution of 1/100 immunoprecipitating mTOR in HeLa whole cell lysate.

Lane 1 (input): HeLa whole cell lysate (10µg)

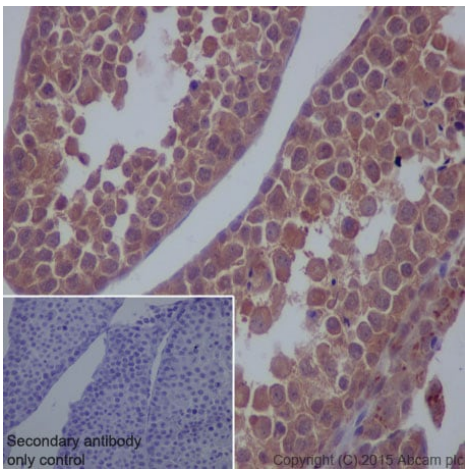
Lane 2 (+): ab32028 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32028 in HeLa whole cell lysate.

For western blotting, **ab131366** VeriBlot for IP Detection Reagent (HRP) was used for detection (1/1000).

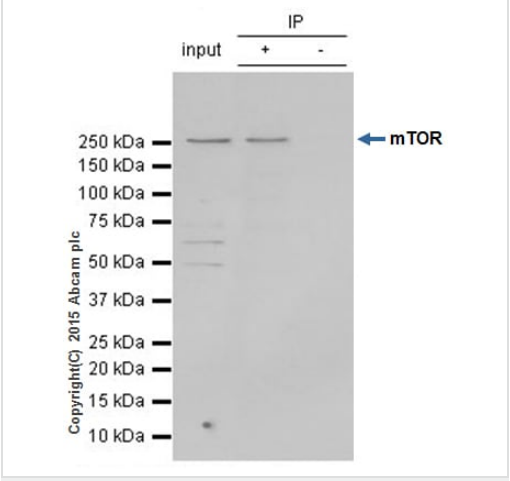
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR antibody [Y391] (ab32028)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse testis tissue labelling mTOR with purified ab32028 at a dilution of 1/400. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunoprecipitation - Anti-mTOR antibody [Y391] (ab32028)

ab32028 (purified) at a dilution of 1/100 immunoprecipitating mTOR in rat brain tissue lysate.

Lane 1 (input): Rat brain tissue lysate (10µg)

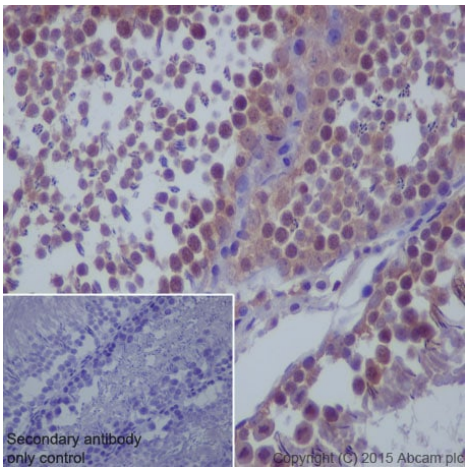
Lane 2 (+): ab32028 + rat brain tissue lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32028 in rat brain tissue lysate.

For western blotting, **ab131366** VeriBlot for IP Detection Reagent (HRP) was used for detection (1/1000).

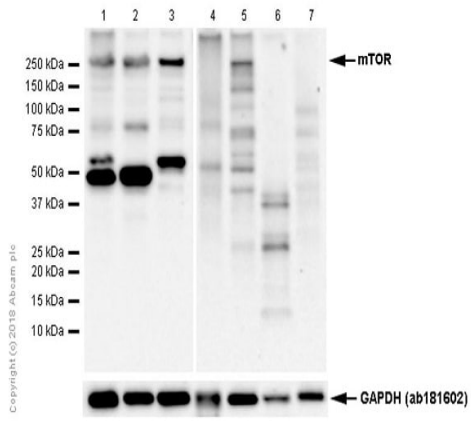
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mTOR antibody [Y391] (ab32028)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat testis tissue labelling mTOR with purified ab32028 at a dilution of 1/400. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-mTOR antibody [Y391] (ab32028)

All lanes : Anti-mTOR antibody [Y391] (ab32028) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with 5% NFDM/TBST
Lane 2 : HaCaT (Human skin keratinocyte) whole cell lysate with 5% NFDM/TBST
Lane 3 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate with 5% NFDM/TBST
Lane 4 : Mouse thymus (8-10 weeks) tissue lysate with 5% NFDM/TBST
Lane 5 : Mouse lung (8-10 weeks) tissue lysate with 5% NFDM/TBST
Lane 6 : Rat thymus tissue lysate with 5% NFDM/TBST
Lane 7 : Rat lung tissue lysate with 5% NFDM/TBST

Lysates/proteins at 20 µg per lane.

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

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

Exposure time:

Lane 1-3: 100 seconds

Lane 4-7: 180 seconds

This antibody detects non-specific bands and high background. It doesn't detect the target band in some mouse and rat tissues.

Anti-mTOR antibody [Y391] (ab32028)

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