# Product datasheet

## Anti-mTOR antibody [Y391] ab32028

**Overview**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product name</strong></td>
<td>Anti-mTOR antibody [Y391]</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit monoclonal [Y391] to mTOR</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
</tr>
</tbody>
</table>
| **Tested applications** | Suitable for: ChIP, WB, IP, IHC-P  
Unsuitable for: Flow Cyt or ICC/IF |
| **Species reactivity** | Reacts with: Mouse, Rat, Human |
| **Immunogen**     | Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human mTOR aa 2400-2500 (C terminal). The exact sequence is proprietary.  
(Peptide available as ab193663) |
| **Epitope**       | ab32028 reacts with an epitope located in the C terminal region of mTOR. |
| **Positive control** | WB: HeLa, Jurkat and Raw264.7 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 cell lysate. |
| **General notes** | Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.  
This product is a recombinant rabbit monoclonal antibody. |

**Properties**

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<tbody>
<tr>
<td><strong>Form</strong></td>
<td>Liquid</td>
</tr>
</tbody>
</table>
| **Storage instructions** | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.  
Avoid freeze / thaw cycle. |
| **Storage buffer** | pH: 7.20  
Preservative: 0.01% Sodium azide  
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA |
| **Purity**    | Protein A purified |
Clonality: Monoclonal
Clone number: Y391
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab32028 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChIP</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 20233713</td>
</tr>
<tr>
<td>WB</td>
<td>!★★★★☆☆☆☆</td>
<td>1/1000 - 1/5000. Detects a band of approximately 250 kDa (predicted molecular weight: 289 kDa). Can be blocked with mTOR peptide (ab193663).</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>1/50 - 1/100.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>!★★★★☆☆☆☆</td>
<td>1/400. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.</td>
</tr>
</tbody>
</table>

Application notes:

Is unsuitable for Flow Cyt or ICC/IF.

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 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/P4-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**

**Images**

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.
All lanes: Anti-mTOR antibody [Y391] (ab32028) at 1/5000 dilution (purified)

Lane 1: HeLa whole cell lysate
Lane 2: Jurkat whole cell lysate
Lane 3: Rat brain tissue lysate
Lane 4: Raw264.7 whole cell lysate

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: 289 kDa

Blocking and dilution buffer: 5% NFDM/TBST

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 (HRP) was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.
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.

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 (HRP) was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.
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.

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