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Product datasheet

Mouse mTOR ELISA Kit ab206311

Recombinant SimpleStep ELISA

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Overview

Product name Mouse mTOR ELISA Kit

Detection method Colorimetric

Precision Intra-assay

Sample	n	Mean	SD	CV%
cell lysate	8			= 3.1%

Inter-assay

Sample	n	Mean	SD	CV%	
cell lysate	3			= 6.9%	

Sample type Cell culture supernatant, Cell culture extracts, Tissue Extracts, Tissue Homogenate

Assay type Sandwich (quantitative)

Sensitivity 16.7 pg/ml

39.06 pg/ml - 2500 pg/ml Range

Recovery Sample specific recovery

Sample type	Average %	Range
Cell culture media	97	94% - 101%

Assay time 1h 30m

Assay duration One step assay

Species reactivity Reacts with: Mouse

Product overview Mouse mTOR ELISA Kit (ab206311) is a single-wash 90 min sandwich ELISA designed for the

> quantitative measurement of mTOR protein in cell culture supernatant, tissue extracts, tissue homogenate, and cell culture extracts. It uses our proprietary SimpleStep ELISA® technology.

Quantitate Mouse mTOR with 16.7 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is

recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (<u>ab203359</u>) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase part of two distinct signaling complexes, mTORC1 and mTORC2. These two complexes share four proteins (mTOR, mLST8, DEPTOR, Tti1/tel2), with only mTORC1 containing Raptor and PRAS40 and mTORC2 containing Rictor, mSin1 and Protor1/2. The complex mTORC1 (rapamycin sensitive complex) coordinates inputs from growth factors, stress, energy status, oxygen and amino acids levels to control processes such as protein and lipid synthesis and autophagy. The complex mTORC2 is insensitive to nutrients and rapamycin, but it responds to insulin signaling. It also controls ion transport and cell shape by targeting serum/glucocorticoid protein kinase (SGK1) and protein kinase (PKC- α) respectively.

The canonical regulation of mTORC1 occurs through the TSC/Rheb pathway which receives signals from AKT, AMPK and IKKβ to activate the complex. Phosphorylation of mTOR at Ser2448 is carried out directly by AKT kinase as well as p70S6 kinase acting as a feedback signal. Phosphorylation at this site is a biomarker for the activation state of the PI-3 kinase pathway as well as the activation status of mTOR. Activation of mTOR leads to phosphorylation of PRAS40, raptor and DEPTOR and the consequential activation of mTORC1. Deregulated signaling of mTOR has been implicated in diseases such as cancer, metabolic syndrome, neurodegeneration and aging. Constitutive activation of PI3K-mTORC1 signaling in cancer cells inhibits autophagy, deregulates protein synthesis via 4E-BP1/eIF4E and increases de novo lipid synthesis via SREBP1. Similarly mTOR signaling is a key factor in the regulation of tissue metabolism in the normal and nutrient overload state affecting the hypothalamus, adipose tissue, the liver, skeletal muscle and pancreas.Notably, rat and human mTOR are 99.5% and 98.9% identical to mouse mTOR, respectively.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Pre-coated microplate (12 x 8 well strips)

Platform

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Wash Buffer PT (ab206977)	1 x 20ml

Notes

Components	1 x 96 tests
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 4BI	1 x 6ml
Mouse mTOR Capture Antibody (lyophilized)	1 vial
Mouse mTOR Detector Antibody (lyophilized)	1 vial
Mouse mTOR Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

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 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 Ill-repressive function. mTORC2 is also activated by growth. factors, but seems to be nutrientinsensitive. 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.

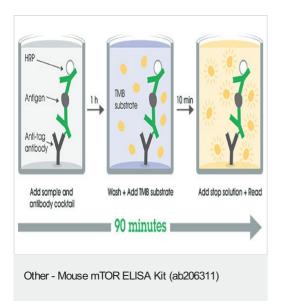
Post-translational modifications

Autophosphorylated; when part of mTORC1 or mTORC2.

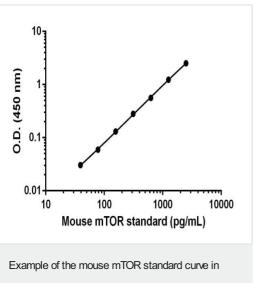
Cellular localization

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.

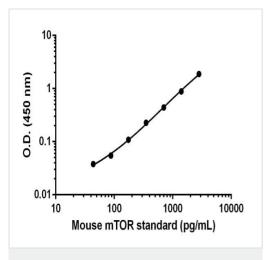
Images



SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

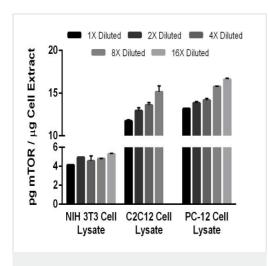


Background-subtracted data values (mean +/- SD) are graphed.



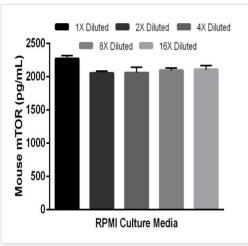
Background-subtracted data values (mean +/- SD) are graphed.

Example of the mouse mTOR standard curve in 1X Cell Extraction Buffer PTR.



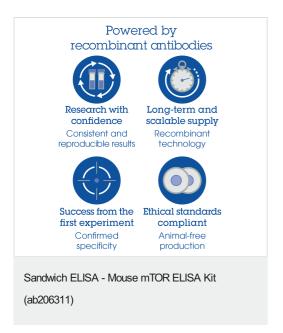
Native linearity of dilution mTOR in cell extracts.

Native mouse mTOR was measured in 600 μ g/mL NIH 3T3 cell extractand 200 μ g/mL C2C12 cell extractdiluted in a 2-fold dilution series in 1X Cell Extraction Buffer PTR. Native rat mTOR was measured in 200 μ g/mL PC-12 cell extractdiluted in a 2-fold dilution series in 1X Cell Extraction Buffer PTR. The concentrations of mouse and rat mTOR were measured in duplicate and interpolated from the mousemTOR standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD).



Linearity of dilution of mouse mTOR in RPMI culture

Recombinant mouse mTOR was spiked into 10% RPMI culture media and diluted in a 2-fold dilution series in Sample Diluent NS. The concentrations of mTOR were measured in duplicate and interpolated from the mouse mTOR standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD).



To learn more about the advantages of recombinant antibodies see **here**.

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