ab206311 – mTOR Mouse SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of mTOR in mouse cell culture supernatants, cell and tissue extracts.

This product is for research use only and is not intended for diagnostic use.

Version 2 Last Updated 3 December 2021

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1. BACKGROUND

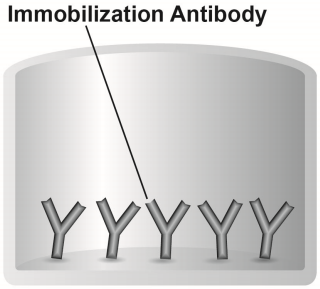
mTOR *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of mTOR protein in mouse cell culture supernatants, cell and tissue extracts.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

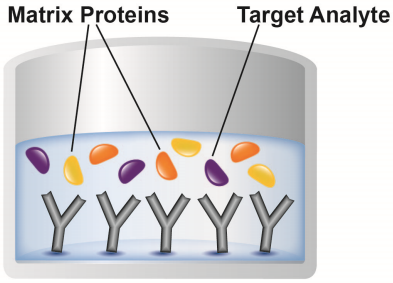
Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase part of two distinct signaling complexes, mTORC1 and mTORC2. These two complexes share four proteins (mTOR, mLST8, DEPTOR, Tti1/tel2), with only mTORC1 containing Raptor and PRAS40 and mTORC2 containing Rictor, mSin1 and Protor1/2. 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.

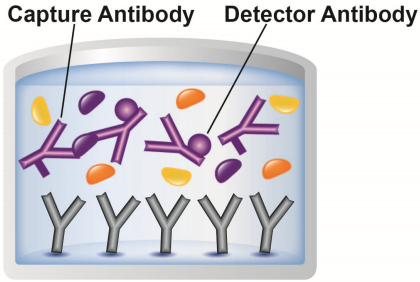
2. ASSAY SUMMARY



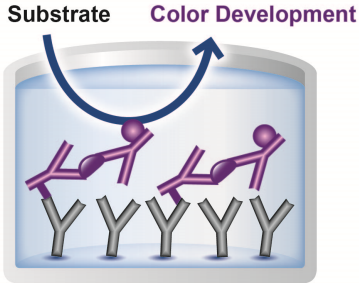
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent and Standard Preparation sections.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Mouse mTOR Capture Antibody (lyophilized)	1 Vial	+2-8ºC
Mouse mTOR Detector Antibody (lyophilized)	1 Vial	+2-8ºC
Mouse mTOR Lyophilized Recombinant Standard Protein	2 Vials	+2-8ºC
Antibody Diluent 4BI	6 mL	+2-8ºC
10X Wash Buffer PT	20 mL	+2-8ºC
5X Cell Extraction Buffer PTR	10 mL	+2-8ºC
50X Cell Extraction Enhancer Solution	1 mL	+2-8ºC
TMB Substrate	12 mL	+2-8ºC
Stop Solution	12 mL	+2-8ºC
Sample Diluent NS	50 mL	+2-8ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8ºC
Plate Seal	1	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps
- When generating positive control samples, it is advisable to change pipette tips after each step.
- The provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4ºC. To dissolve, warm briefly at + 37ºC and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only)

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

9.2 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.3 10X Capture antibody

Prepare the 10X Capture Antibody solution by adding 330 µL distilled H_2O and 330 µL Sample Diluent NS to the lyophilized Capture Antibody vial. Mix thoroughly and gently on a tube rotator at room temperature for 10 minutes, then keep on ice. Store any un-used 10X Capture Antibody at -20ºC.

9.4 **10X Detector antibody**

Prepare the 10X Detector Antibody solution by adding 330 µL distilled H_2O and 330 µL Sample Diluent NS to the lyophilized Detector Antibody vial. Mix thoroughly and gently on a tube rotator at room temperature for 10 minutes, then keep on ice.

Store any un-used 10X Detector Antibody at -20ºC.

9.5 **Antibody Cocktail**

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following section describes the preparation of a standard curve for duplicate measurements (recommended).

The mouse mTOR protein standard included in this kit is a recombinant fragment from AA1 – AA200. This fragment is 22.1 kDa in size.

IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the mTOR standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the mTOR standard by adding 1 mL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 15,000 pg/mL **Stock Standard** Solution.

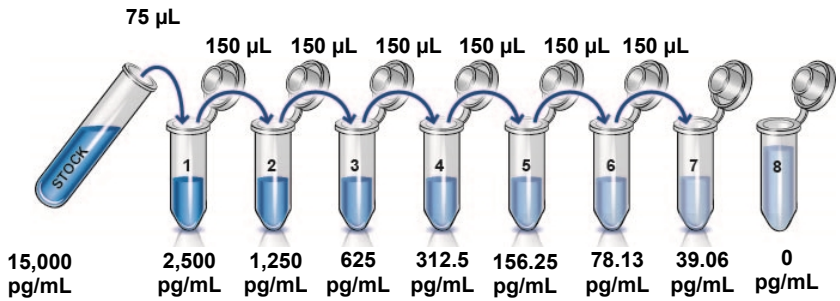
10.1 For **cell culture supernatant samples**, follow these instructions.

10.1.1 Reconstitute the mTOR standard by adding Sample Diluent NS by pipette.

10.1.2 Label eight tubes, Standards 1– 8.

10.1.3 Add 375 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.

10.1.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



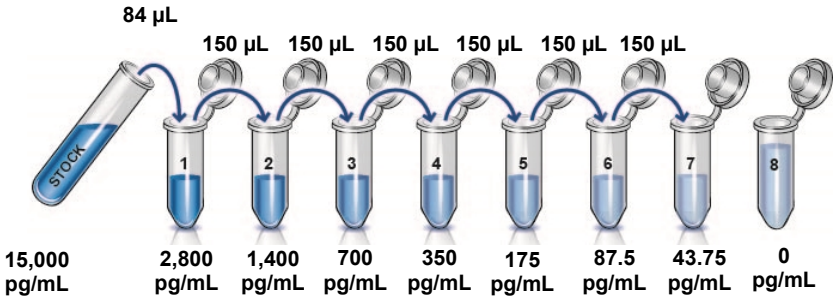
10.2 For **cell and tissue extract samples**, follow these instructions:

10.2.1 Reconstitute the mTOR standard by adding 1X Cell Extraction Buffer PTR by pipette.

10.2.2 Label eight tubes, Standards 1– 8.

10.2.3 Add 366 µL Sample Diluent 1X Cell Extraction Buffer PTR into tube number 1 and 150 µL of Sample Diluent 1X Cell Extraction Buffer PTR into numbers 2-8.

10.2.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
NIH 3T3 Cell Lysate	37.5 µg/mL – 600 µg/mL
C2C12 Cell Lysate	25 µg/mL – 200 µg/mL
PC-12 Cell Lysate	12.5 µg/mL – 200 µg/mL
RPMI Base Media	1:10 – 1:160

11.1 Cell Culture Supernatants

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and dilute samples into Sample Diluent NS and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.2 Preparation of extracts from cell pellets

11.2.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC.

11.2.2 Rinse cells twice with PBS.

11.2.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.2.4 Incubate on ice for 20 minutes.

11.2.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.2.6 Transfer the supernatants into clean tubes and discard the pellets.

11.2.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.2.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.3 **Preparation of extracts from adherent cells by direct lysis (alternative protocol)**

11.3.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.3.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.3.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.3.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.3.5 Transfer the supernatants into clean tubes and discard the pellets.

11.3.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.3.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.4 **Preparation of extracts from tissue homogenates**

11.4.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.4.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.4.3 Incubate on ice for 20 minutes.

11.4.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.4.5 Transfer the supernatants into clean tubes and discard the pellets.

11.4.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.4.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C
- For each assay performed, a minimum of two wells must be used as the zero control
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Differences in well absorbance or “edge effects” have not been observed with this assay

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.9 Analyze the data as described below.

14. CALCULATIONS

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

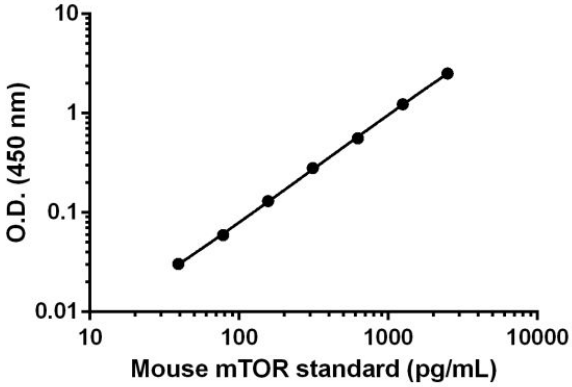
14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 The interpolated value obtained with this kit corresponds to the equivalent value of the mTOR recombinant protein fragment, which is 22.11kDa in size. To determine the actual concentration of the native mTOR in cell and tissue extracts, the interpolated value should be multiplied by the correction factor 13.6, as the full length protein is 13.6 times larger than the fragment provided (288.78kDa)

14.5 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

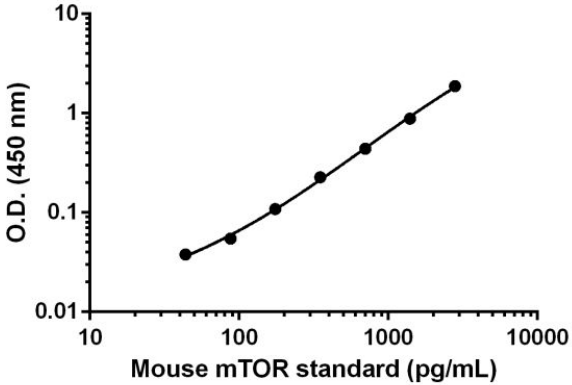
15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.064	0.068	0.066
39.06	0.097	0.095	0.096
78.13	0.127	0.124	0.125
156.25	0.197	0.196	0.196
312.5	0.360	0.336	0.348
625	0.635	0.616	0.626
1,250	1.319	1.277	1.298
2,500	2.682	2.491	2.587

Figure 1. Example of the mouse mTOR standard curve in Sample Diluent NS. The m mTOR standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.060	0.066	0.063
43.75	0.101	0.101	0.101
87.5	0.114	0.122	0.118
175	0.171	0.172	0.172
350	0.276	0.307	0.291
700	0.497	0.513	0.505
1,400	0.936	0.957	0.946
2,800	1.925	1.950	1.937

Figure 2. Example of the mouse mTOR standard curve in 1X Cell Extraction Buffer PTR. The mouse mTOR standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. CALIBRATION

This immunoassay is calibrated against a highly purified mouse mTOR fragment aa1 – aa200. The dose response curve of mTOR native protein on NIH3T3, C2C12 and PC-12 cell lysates parallels the SimpleStep standard curve (see linearity of dilution below). To convert sample values obtained with the SimpleStep mouse mTOR kit to approximate native mTOR concentration, use the equation below.

Full length (mTOR) approximate value (ng/mL) = 13.6 x SimpleStep mouse mTOR value (ng/mL)

17. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable dose (MDD) is determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentrations. The MDD is dependent on the Sample Diluent buffer used:

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	24	16.7 pg/mL
1X Cell Extraction Buffer PTR	19	30.7 pg/mL

RECOVERY –

Three concentrations of mouse mTOR were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
10% RPMI Culture Media	97	94 - 101

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native mTOR was measured in NIH 3T3, C2C12, and PC-12 cell lysates in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Recombinant mouse mTOR was spiked into RPMI culture media and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	600 µg/mL NIH 3T3 Extract	200 µg/mL C2C12 Extract	200 µg/mL PC-12 Extract	10% RPMI Culture Media
Undiluted	pg/mL	2483.5	2348.4	2636.1	2271.8
	% Expected value	**100**	**100**	**100**	**100**
2	pg/mL	1481.1	1293.9	1383.6	1026.7
	% Expected value	**119**	**110**	**105**	**90**
4	pg/mL	680.6	679.6	708.3	514.3
	% Expected value	**110**	**116**	**107**	**91**
8	pg/mL	360.4	366.5	394.1	261.9
	% Expected value	**116**	**125**	**120**	**92**
16	pg/mL	197.2	NL	206.5	131.7
	% Expected value	**127**	**NL**	**125**	**93**

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of NIH 3T3 lysate and 3 concentrations of PC-12 lysate within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	8	3
CV (%)	3.1	6.9

SAMPLE VALUES –

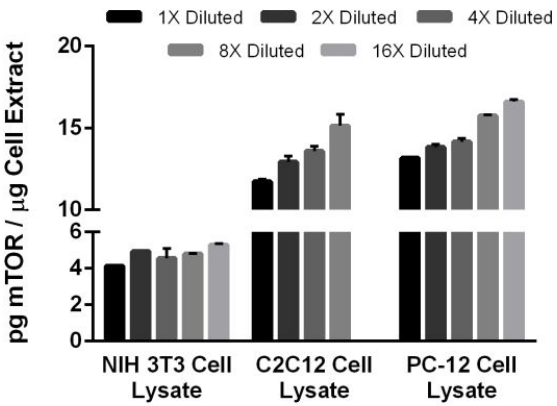


Figure 3. Native linearity of dilution mTOR in cell extracts. Native mouse mTOR was measured in 600 µg/mL NIH 3T3 cell extract and 200 µg/mL C2C12 cell extract diluted in a 2-fold dilution series in 1X Cell Extraction Buffer PTR. Native rat mTOR was measured in 200 µg/mL PC-12 cell extract diluted in 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 mouse mTOR standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD).

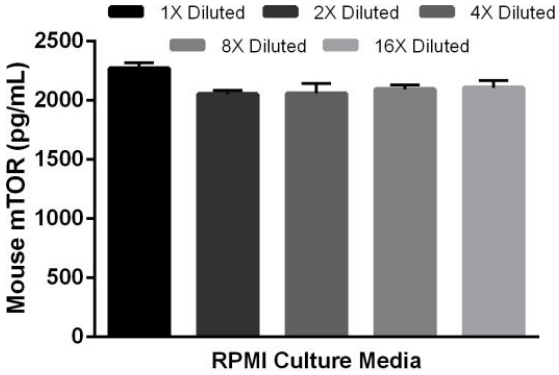


Figure 4. Linearity of dilution of mouse mTOR in RPMI culture media. Recombinant mouse mTOR was spiked into 10% RPMI culture media and diluted in a 2-fold dilution series in Sample Diluent NS. The concentrations of mTOR were measured in duplicate and interpolated from the mouse mTOR standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD).

18. SPECIES REACTIVITY

This kit reacts with mouse, rat and human mTOR.

Please contact our Technical Support team for more information

19. TROUBLESHOOTING

Problem	Cause	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

20. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)