

Datasheet for 600-401-422**mTOR phospho S2448 Antibody****Overview**

Description:	Anti-mTOR pS2448 (RABBIT) Antibody - 600-401-422
Item No.:	600-401-422
Size:	100 µg
Applications:	ELISA, IHC, WB, IF
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Mammalian target of rapamycin (mTOR) is a serine and threonine protein kinase that regulates numerous cellular functions, in particular, the initiation of protein translation. Rapamycin is a natural product macrolide that induces G ₁ growth arrest in yeast, Drosophila, and mammalian cells. mTOR has a long list of synonyms including FK506 binding protein12 - rapamycin associated protein 1, FK506 binding protein12 - rapamycin associated protein 2, FRAP1, FRAP2, RAFT1, RAPT1 and/or FKBP12-rapamycin associated protein (FRAP). mTOR is one of a family of proteins involved in cell cycle progression, DNA recombination, and DNA damage detection. In rat, mTOR is a 245-kD protein referred to as RAFT1 with significant homology to the Saccharomyces cerevisiae protein TOR1 and has been shown to associate with the immunophilin FKBP12 in a rapamycin-dependent fashion. The FKBP12-rapamycin complex is known to inhibit progression through the G ₁ cell cycle stage by interfering with mitogenic signaling pathways involved in G ₁ progression in several cell types, as well as in yeast. The binding of mTOR to FKBP12-rapamycin correlates with the ability of these ligands to inhibit cell cycle progression.
Synonyms:	rabbit anti-mTOR pS2448 antibody, FKBP12 rapamycin complex associated protein antibody, Serine/threonine-protein kinase mTOR, FK506-binding protein 12-rapamycin complex-associated protein 1, Mammalian target of rapamycin, mTOR, Mechanistic target of rapamycin, Rapamycin and FKBP12 target 1, Rapamycin target protein 1, FRAP, FRAP1, FRAP2, RAFT1, RAPT1
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	MTOR
Reactivity:	Human
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-mTOR pS2448 antibody was prepared from whole rabbit serum produced by repeated immunizations with a phosphorylated synthetic peptide corresponding a c-terminal region near Serine 2448 of human mTOR.
Purity/Specificity:	This is an affinity purified antibody produced by immunoaffinity chromatography using the immunizing peptide after immobilization to a solid phase. Reactivity occurs with phosphorylated mTOR from human derived tissues and cells. Reactivity against mTOR from other species has not been determined, however, reactivity with mouse and rat is suggested based on protein sequence homologies.
Relevant Links:	• NCBI - 1169735 • UniProtKB - P42345 • GenelD - 2475

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IF (Based on references)
Application Note:	This affinity purified antibody has been tested for use in immunohistochemistry, ELISA and western blotting. Western blotting shows reactivity specific for phospho mTOR detecting a band at approximately 250 kDa. Reactivity in other immunoassays is unknown.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:100,000
IHC:	5.0 µg/ml
WB:	1:500 - 1:2,000

Formulation

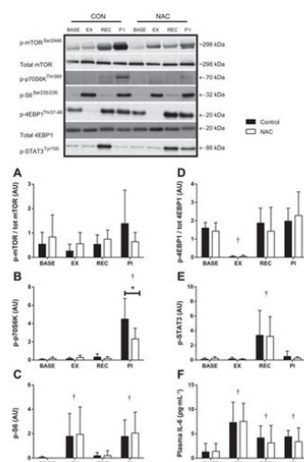
Physical State:	Liquid (sterile filtered)
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Concentration:	1.1mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

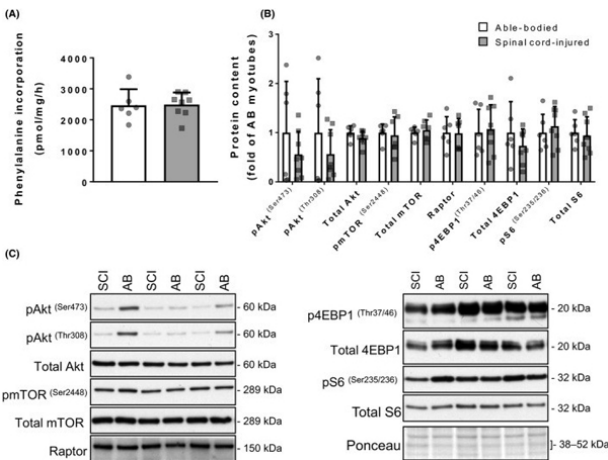
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



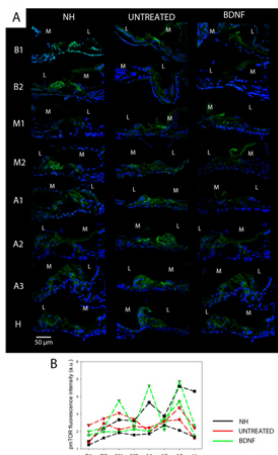
Western Blot

Effect of exercise and insulin stimulation with NAC or CON infusion on phosphorylation of protein translation regulation proteins mTOR Ser2448 (A), p70S6K Thr389 (B), S6 Ser235/236 (C), eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) Thr37/46 (D), and STAT3 Tyr705 (E). Western blots normalized to Ponceau S stain for each lane, and representative blots from 1 participant are shown. Data are means $\pm$ SD for $n = 7$. *Significantly different, NAC vs. CON ($P < 0.05$); †significantly different from BASE ($P < 0.05$). Fig 6. PMID: 26105008



Western Blot

Protein synthesis in differentiated myotubes. (A) Phenylalanine incorporation into protein in myotubes from spinal cord-injured (gray bars) and able-bodied (white bars) individuals. Bars represent mean $\pm$ SD and individual data points are overlaid. (B) Phosphorylated and total protein content of molecules in the Akt-mTOR signalling axis (pAkt (Ser473), pAkt(Thr308), total Akt, mTOR (Ser2448), total mTOR, Raptor, p4EBP1(Ser37/46), total 4EBP, pS6(Ser235/236), total S6) in myotubes from spinal cord-injured (gray bars) and able-bodied (white bars) individuals. Bars represent mean $\pm$ SD and individual data points are overlaid. n = 6–8; Mann–Whitney test (significance $P < 0.05$). (C) Representative Western blot images. mTOR, mechanistic target of rapamycin; 4EBP, 4E binding protein. Fig 3. PMID: 29906337

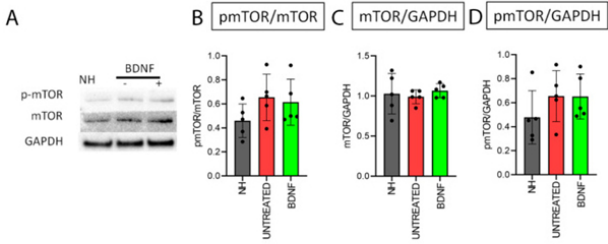


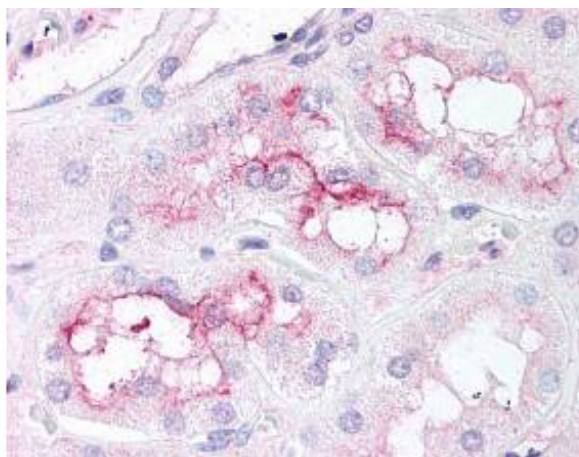
Immunofluorescence Microscopy

Immunolocalization and fluorescence intensity of pmTOR. (A) Representative confocal images showing the organ of Corti immunolabelled with anti-pmTOR (green) and counterstained with bisbenzimidazole nuclear dye (blue) of all experimental groups and for each cochlear location (from B1 to H). Scale bar: 50 μ m. L: lateral, M: medial. (B) Fluorescence intensity analysis of pmTOR immunostaining. The graph shows the signal intensity of each sample for all cochlear locations (black: NH, red: untreated, green: BDNF-treated). The same symbol was used to identify the BDNF-treated and untreated ears of individual animals. Fig 8. PMID: 36428503

Western Blot

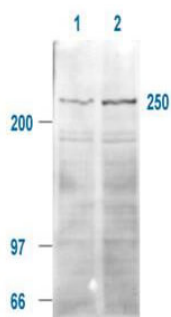
Protein quantification of pmTOR (p/n 600-401-422) and mTOR (p/n 600-401-897). (A) Representative Western blot bands (original Western blot bands are shown in Supplementary Figure S2). Western blot analysis of pmTOR/mTOR (B), mTOR/GAPDH (C) and pmTOR/GAPDH (D) on organ of Corti samples from all the experimental groups. Histograms show mean $\pm$ SD; the dots indicate the densitometric values for individual samples (n = 5; each sample is a pool of two organs of Corti). Fig 11. PMID: 36428503





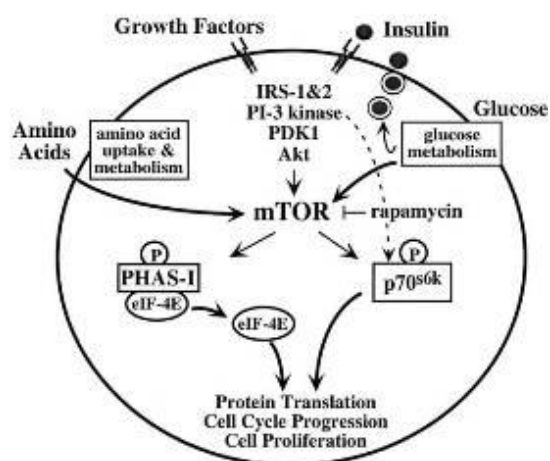
Immunohistochemistry

Rockland's affinity purified anti-mTOR pS 2448 antibody was used at 5 µg/ml to detect signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows moderate staining of proximal convoluted tubules of the kidney (40X). Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.



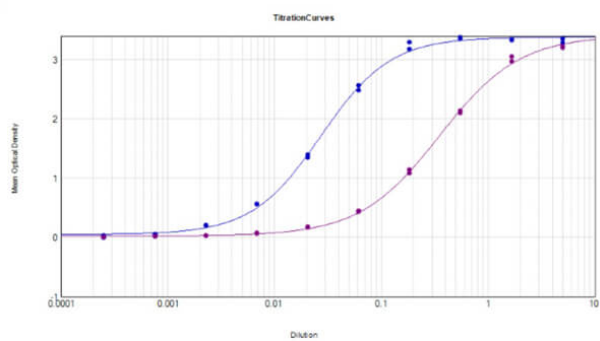
Western Blot

Affinity Purified Anti-mTOR pS 2448 (Rabbit) is shown to detect a 250 kDa band (indicated) corresponding to phosphorylated human mTOR present in a 293T whole cell lysates. Cells were serum-starved for 24 hours prior to harvest. ~20 µg of lysate was loaded per lane for SDS-PAGE. Untreated cells are shown in lane 1, whereas cells in lane 2 were treated with IGF-1 (100 ng/ml) for 20 min prior to harvest. Follow reaction of antibody with a 1:2000 dilution of HRP Goat-a-Rabbit IgG for visualization.



Pathway

Metabolic and autocrine regulation of the mTOR pathway by Beta-cells.



ELISA

ELISA Results of Rabbit Anti-mTOR pS2448 Antibody tested against BSA-conjugated non-phospho [purple line] and phospho [blue line] forms of immunizing peptide. Each well was coated in duplicate with either 0.1µg of conjugate. The working dilution is 1:37,000. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using HRP conjugate stabilizer (p/n MB-067), Goat Anti-Rabbit HRP conjugated (p/n 611-103-122) and TMB substrate (p/n TMBE-1000).

References

- Tisi A et al. mTOR Signaling in BDNF-Treated Guinea Pigs after Ototoxic Deafening. *Biomedicines*. (2022)
- Savikj et al. Retained differentiation capacity of human skeletal muscle satellite cells from spinal cord-injured individuals. *Physiological Reports* (2018)
- Trewin AJ et al. Effect of N-acetylcysteine infusion on exercise-induced modulation of insulin sensitivity and signaling pathways in human skeletal muscle. *Am J Physiol Endocrinol Metab*. (2015)

Disclaimer

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