

Datasheet for 600-401-897**mTOR Antibody****Overview**

Description:	Anti-mTOR (RABBIT) Antibody - 600-401-897
Item No.:	600-401-897
Size:	100 µg
Applications:	ELISA, 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
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to an internal region near amino acids 2430-2460 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 both phosphorylated and non-phosphorylated forms of mTOR at S2448 from human derived tissues and cells. A BLAST analysis was used to suggest cross reactivity with mTOR protein from rat and mouse based on 100% homology with the immunizing sequence. Expect partial reactivity against mTOR homologues from zebrafish (94%) and dog (89%). Reactivity against homologues from other sources is not known.
Relevant Links:	• NCBI - 1169735 • UniProtKB - P42345 • GeneID - 2475

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IF (Based on references)
Application Note:	This affinity purified mTOR antibody has been tested for use in ELISA and western blotting. ELISA data demonstrate reactivity against both phosphorylated and non-phosphorylated mTOR at S2448 and western blotting shows 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:	1:50,000 - 1:100,000
WB:	1:250 - 1:2,000

Formulation

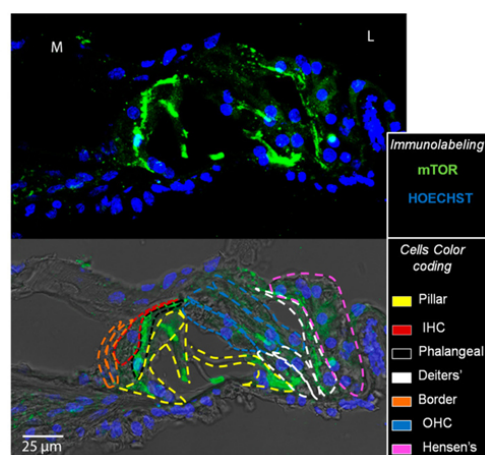
Physical State:	Liquid (sterile filtered)
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Concentration:	0.9 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

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.

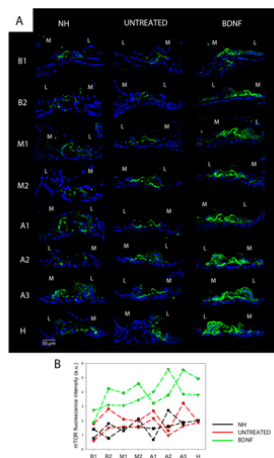
Images



Immunofluorescence Microscopy

Immunolocalization of the mTOR protein. Top: a representative confocal image of the organ of Corti of a NH cochlea (A1 location) immunolabelled with anti-mTOR (green) and counterstained with bisbenzimidazole nuclear dye (blue). Bottom: The same image was acquired with a bright-field background to visualize the structure of the organ of Corti; the different cell types were signed with color coding (pillar: yellow, IHC: red, phalangeal: black, Deiters': white, border: orange, OHC: blue, Hensen's: pink). L: lateral, M: medial. Fig 9.

PMID: 36428503



Immunofluorescence Microscopy

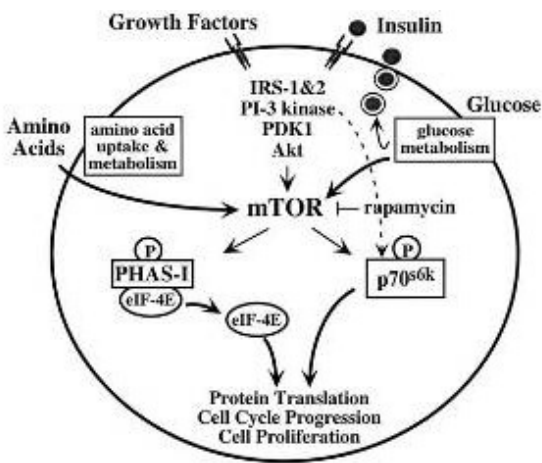
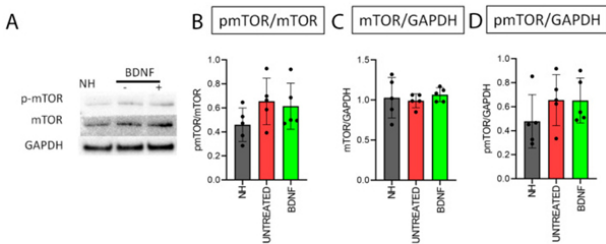
Immunolocalization and fluorescence intensity of mTOR. (A) Representative confocal images showing the organ of Corti immunolabelled with anti-mTOR (green) and counterstained with bisbenzimidazole nuclear dye (blue) of all experimental groups and for each cochlear location (from B1 to H). Scale bar: 50 μ m. L: lateral, M: medial. (B) Fluorescence intensity analysis of mTOR 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 10.

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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.

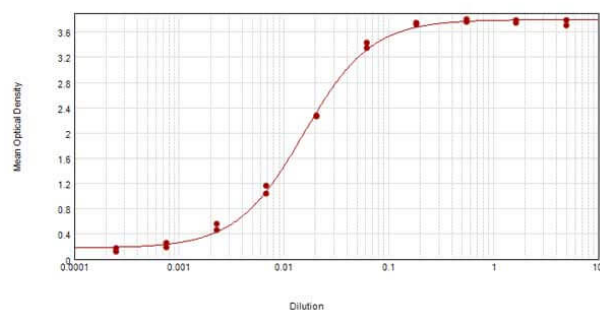
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Pathway

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

Anti-mTOR Sensitivity

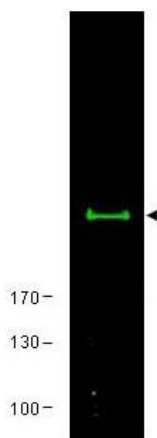


ELISA

ELISA results of purified Rabbit anti-mTOR Antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1 µg of conjugate. The starting dilution of antibody was 5 µg/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 3% fish gel (p/n MB-066), Goat anti-Rabbit IgG Antibody Peroxidase Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) (p/n 611-103-122) and TMB ELISA Peroxidase Substrate (p/n TMBE-1000).

Western Blot

Western blot using Rockland's Affinity Purified anti-mTOR antibody shows detection of a band ~245 kDa corresponding to human mTor (arrowhead). Approximately 30 µg of HEK293 cell lysate (p/n W09-000-365) was separated by 4-8% SDS-PAGE and transferred onto nitrocellulose. After blocking, the membrane was probed with the primary antibody diluted to 1:650 for 2h at RT. The membrane was washed and reacted with a 1:10,000 dilution of IRDye™800 conjugated Gt-a-Rabbit IgG [H&L] MX (p/n 611-132-122) for 45 min at room temperature. IRDye™800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.



References

- Tisi A et al. mTOR Signaling in BDNF-Treated Guinea Pigs after Ototoxic Deafening. *Biomedicines*. (2022)

Disclaimer

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