ROCKLAND
Immunochemicals for Research

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Certificate of Analysis

Product: Affinity Purified Anti-mTOR (Rabbit)

Code: 600-401-897 **Lot #:** 18342

Size: 100 μg **Physical State:** Liquid (sterile filtered)

Antibody Concentration: 0.40 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None **Preservative:** 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

Background Information: 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.

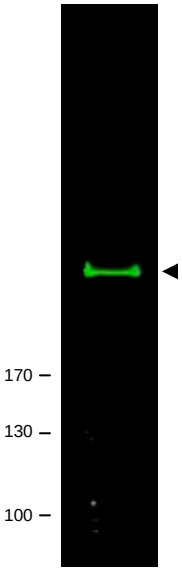


Figure 1. Western blot using Rockland's Affinity Purified anti-mTOR antibody shows detection of a band ~245 kDa corresponding to human mTor (arrowhead). Approximately 30 μg of HEK293 cell lysate was separated by 4-8% SDS-PAGE and transferred onto nitrocellulose. After blocking, the membrane was probed 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 (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.

Application Note(s): This affinity purified antibody is suitable 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.

Recommended Dilutions:

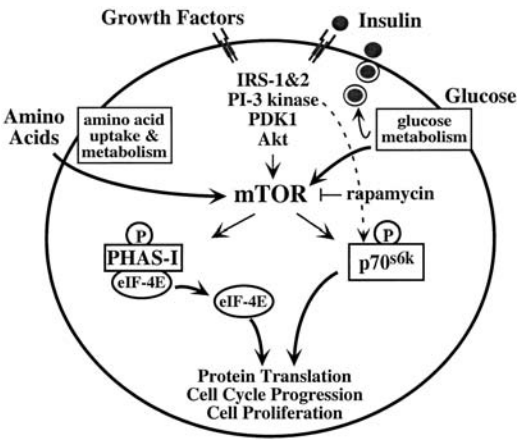
ELISA	1:4,000 - 1:20,000
WESTERN BLOT	1:250 - 1:2,000
IF MICROSCOPY	User Optimized
OTHER APPLICATIONS	User Optimized

Purity and 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.

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 2440-2457 of human mTOR.

Relevant Links: NCBI P42345 Swiss-Prot P42345

Figure 2. Metabolic and autocrine regulation of the mTOR pathway by β-cells.



General References:

Kristof,A.S. et al. (2003) Stimulation of signal transducer and activator of transcription-1 (STAT1)-dependent gene transcription by lipopolysaccharide and interferon-gamma is regulated by mammalian target of rapamycin. *J. Biol. Chem*. **278** (36), 33637-33644.

Chen,Y., et al. (2003) Phospholipase D confers rapamycin resistance in human breast cancer cells. *Oncogene* **22** (25), 3937-3942.

Nojima,H. et al. (2003) The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif. *J. Biol. Chem*. **278** (18), 15461-15464.

McMahon,L.P. et al. (2002) The rapamycin-binding domain governs substrate selectivity by the mammalian target of rapamycin. *Mol. Cell. Biol.* **22** (21), 7428-7438.

Hudson,C.C. et al. (2002) Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol. Cell. Biol.* **22** (20), 7004-7014.

Choi,J.H. et al. (2002) The FKBP12-rapamycin-associated protein (FRAP) is a CLIP-170 kinase. *EMBO* Rep. **3** (10), 988-994.

Protein Sequence: Human lymphoma (truncated) mTOR, 2549 aa, predicted MW 288.8 kDa

2041	ernvkgmfev	leplhammer	gpqtlketsf	nqaygrdlme	aqewcrkymk	sgnvkdltqa
2101	wdlyyhvfrr	iskqlpqlts	lelqyvspkl	lmcrdlelav	pgtydpnqpi	iriqsiapsl
2161	qvitskqrpr	kltlmgsngh	efvfllkghe	dlrqdervmq	lfglvntlla	ndptslrknl
2221	stqryavipl	stnsgligwv	phcdtlhali	rdyrekkkil	lniehrimlr	mapdydhltl
2281	mqkvfvfeha	vnntagddla	kllwlkspss	evwfdrrtny	trslavmsmv	gyilglgdrh
2341	psnlmldrls	gkilhidfgd	cfevamtrek	fpekipfrlt	rmltnamevt	gldgnyritc
2401	htvmevlreh	kdsvmavlea	fvydpllnwr	lmdtntkgn**k**	**rsrtrtdsys**	**agqsvei**ldg
2461	velgepahkk	tgttvpesih	sfigdglvkp	ealnkkaiqi	inrvrdkltg	rdfshddtld
2521	vptqvellik	qatshenlcq	cyigwcpfw			

Related Products:

#W09-000-365	293 Whole Cell Lysate in SDS-PAGE Sample Buffer
#611-703-127	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
#611-132-122	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#MB-070	Blocking Buffer for Fluorescent Western Blotting
#KIA-003	***Max*Tag**™ Anti-RABBIT IgG Kit for Immunoblotting
#MB-070	Blocking Buffer for Fluorescent Western Blotting

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.