

### Certificate of Analysis

**Product:** Affinity Purified Anti-mTOR pS2448 (Rabbit)

**Code:** 600-401-422

**Lot #:** 18341

**Size:** 100 µg

**Physical State:** Liquid (sterile filtered)

**Antibody Concentration:** 1.50 mg/ml (by UV absorbance at 280 nm)

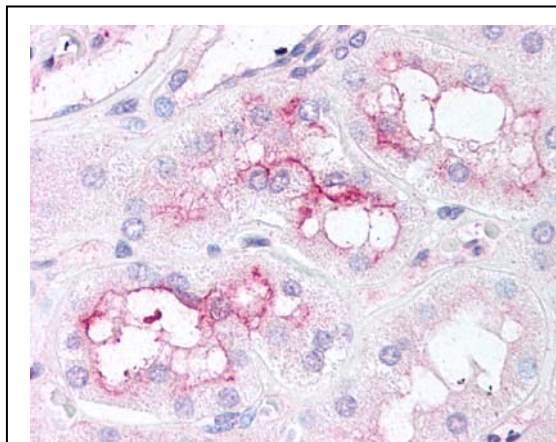
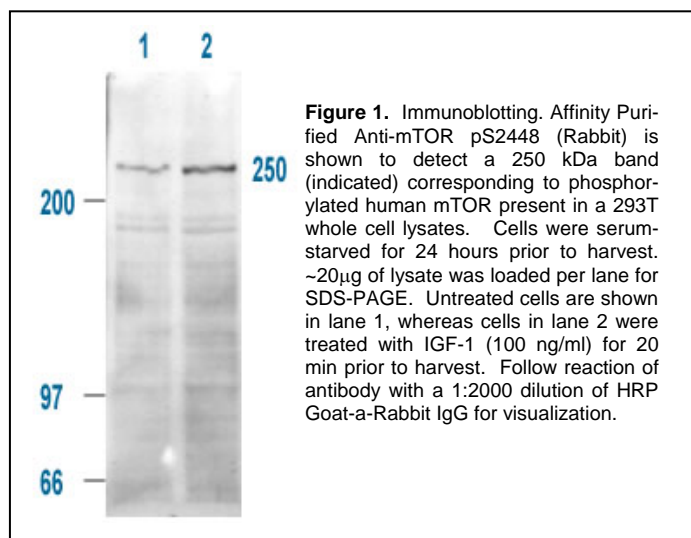
**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

**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.

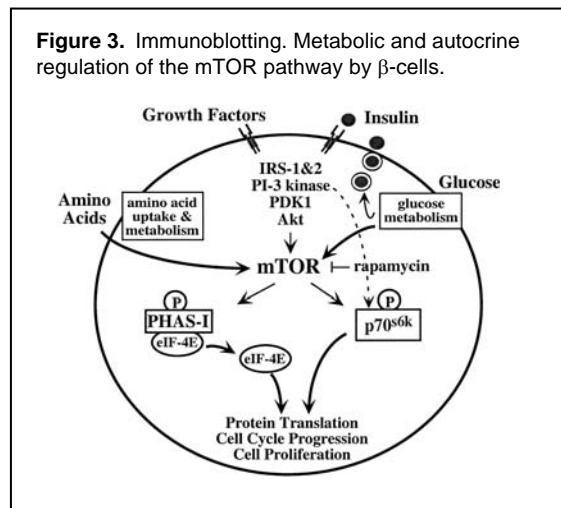
**Background Information:** Mammalian target of rapamycin (mTOR) is a serine and threonine protein kinase that regulates numerous cellular functions, in particular, the initiation of protein translation. Rapamycin is a natural product macrolide that induces G<sub>1</sub> growth arrest in yeast, *Drosophila*, 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<sub>1</sub> cell cycle stage by interfering with mitogenic signaling pathways involved in G<sub>1</sub> 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 2.** Immunohistochemistry. ROCKLAND's affinity purified anti-mTOR pS2448 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. 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.*

**Application Note(s):** 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.

<b>Recommended Dilutions:</b>	<b>ELISA</b>	1:15,000 - 1:65,000
	<b>WESTERN BLOT</b>	1:500 - 1:2,000
	<b>IMMUNOHISTOCHEMISTRY</b>	5.0 µg/ml
	<b>OTHER APPLICATIONS</b>	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 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.

**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:**

ExPASy <http://www.expasy.org/cgi-bin/niceprot.pl?P42345>

**General References:**

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- 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 1 $\alpha$  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.
- Inoki, K. et al. (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat. Cell Biol.* **4** (9), 648-657.
- Zhang, X. et al. (2002) Predominant nuclear localization of mammalian target of rapamycin in normal and malignant cells in culture. *J. Biol. Chem.* **277** (31), 28127-28134.

**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.

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