

Purified anti-mTOR Phospho (Ser2448) Antibody

Catalog# / Size	610301 / 25 µg 610302 / 100 µg
Clone	A17024A
Regulatory Status	RUO
Other Names	Mammalian target of rapamycin, FRAP, RAFT
Isotype	Mouse IgG1, κ
Description	<p>mTOR is a member of the PI3 family of kinases and plays a critical role in regulating cell proliferation and metabolism in response to nutrient availability, cellular energy status, hormones, growth factors, and stress. mTOR is found as a component of two functionally distinct oligomeric complexes, mTORC1 and 2 (mTOR complex 1 and 2). mTORC1 coordinates nutrient availability with cell growth and proliferation through phosphorylation of upstream regulators of proliferation and ribosomal biogenesis including eIF4BP1 and RPS6KB1 and RPS6KB2. Additionally, mTORC1 controls autophagy through negative regulation of an autophagy-promoting kinase complex comprised of ULK1, ATG13, and FIP200. mTORC2 regulates proliferation and metabolism via phosphorylation of IG-1R, InsR, and Akt, and also plays a role in cytoskeletal architecture and reorganization through modification of Rac1.</p> <p>The mTOR subunit of mTORC1 and 2 is phosphorylated at Ser2448 in a PI3/Akt- signaling-dependent manner; this post-translational modification is associated with increased mTORC activity.</p>

Product Details

Reactivity	Human, Mouse
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Preparation	The antibody was purified by affinity chromatography.
Concentration	0.5 mg/ml
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C.
Application	WB - Quality tested ICC - Verified
Recommended Usage	Each lot of this antibody is quality control tested by Western blotting . For Western blotting, the suggested use of this reagent is 0.1 - 1.0 µg per ml. For immunocytochemistry, a concentration of 5 µg/ml is recommended. It is recommended that the reagent be titrated for optimal performance for each application.
Application Notes	<p>Based off complete sequence homology of the immunizing peptide, this clone is predicted to recognize both mouse and rat mTOR phosphorylated at serine 2448.</p> <p>NIH/3T3 cells (murine origin) used in this experiment contain less mTOR than the human-derived HeLa cells (see pan mTOR blot). Thus, the reduced signal observed in these lysates is due to less total mTOR and not a decreased affinity of this clone for mouse mTOR phosphor (Ser2448).</p>
RRID	AB_2801105 (BioLegend Cat. No. 610301) AB_2801106 (BioLegend Cat. No. 610302)

Antigen Details

Structure	mTOR is a 2,549 amino acid protein with a predicted molecular weight of ~289 kD.
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Distribution	Ubiquitously expressed; cytosolic, lysosomal, and nuclear cellular localization
Function	Regulates cell proliferation and metabolism
Interaction	Part of the mammalian target of rapamycin complex 1 (mTORC1) which contains MTOR, MLST8, RPTOR, AKT1S1/PRAS40, and DEPTOR; also part of the mammalian target of rapamycin complex 2 (mTORC2) which contains MTOR, MLST8, PRR5, RICTOR, MAPKAP1, and DEPTOR.
Biology Area	Cell Biology, Cell Motility/Cytoskeleton/Structure, Cell Proliferation and Viability, Protein Synthesis, Signal Transduction
Molecular Family	Phospho-Proteins, Protein Kinases/Phosphatase
Antigen References	<ol style="list-style-type: none"> 1. Brown EJ, <i>et al.</i> 1994. <i>Nature</i>. 369:756-8. 2. Dennis P, <i>et al.</i> 2001. <i>Science</i>. 294:1102. 3. Fingar D, <i>et al.</i> 2002. <i>Genes Dev.</i> 16:1472. 4. Nojima H, <i>et al.</i> 2003. <i>J. Biol. Chem.</i> 278:15461.
Gene ID	2475

Related Protocols

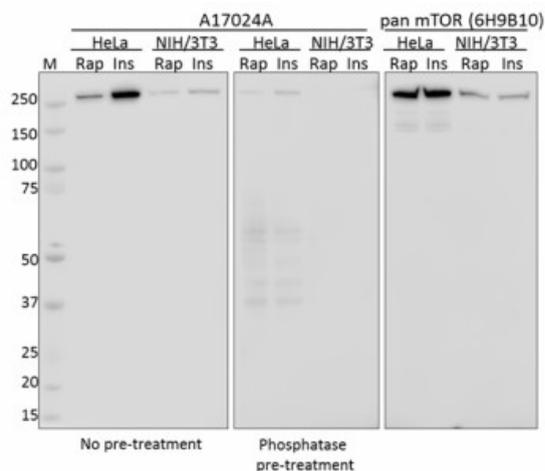
[Immunocytochemistry Staining Protocol](#)

[Western Blotting Protocol](#)

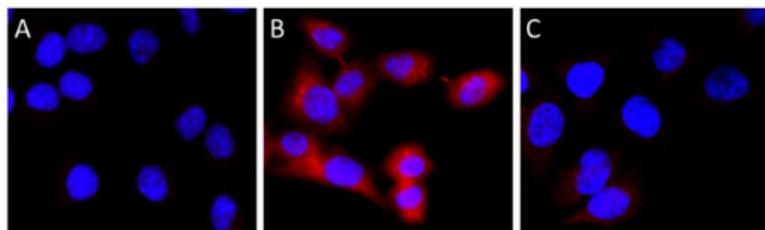
Other Formats

Purified anti-mTOR Phospho (Ser2448)

Product Data



Whole cell extracts (15 µg protein) from serum starved HeLa and NIH/3T3 cells treated with 10 µM rapamycin (Rap) for 2 hours or with 150 nM insulin (Ins) for 6 minutes were resolved by 4-12% Bis-Tris gel electrophoresis, transferred to a nitrocellulose membrane, and probed with 0.5 µg/mL (1:1000 dilution) of purified anti-mTOR Phospho (Ser2448), clone A17024A, for 2 hours at room temperature. To confirm phospho-specificity of A17024A, a duplicate membrane was pre-treated with lambda protein phosphatase prior to incubation with A17024A. Proteins were visualized by chemiluminescence detection using HRP goat anti-mouse-IgG (Cat. No. 405306) at a 1:3000 dilution. Equal mTOR loading was confirmed by probing membranes with an anti-pan mTOR antibody (Cat. No. 659202) at 1.0 µg/mL (1:500 dilution). Lane M: Molecular Weight Marker.



Serum starved HeLa cells treated with 150 nM insulin for 6 minutes were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with ice-cold methanol for 10 minutes, and blocked with 5% FBS for 60 minutes. Cells were then intracellularly stained with a 1:100 dilution (5 µg/mL) of either mouse IgG (panel A) or purified anti-mTOR Phospho (Ser2448) antibody (panel B) for two hours at room temperature, followed by incubation with Alexa Fluor® 594 goat anti-mouse IgG (Cat. No.

405326) at 2.0 µg/mL. To confirm phospho-specificity of A17024A, fixed cells pre-treated with lambda protein phosphatase prior to staining were included as a control (panel C). Nuclei were counterstained with DAPI, and the image was captured with a 60X objective.

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BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587