Store at -20°C

#2971

Phospho-mTOR (Ser2448) Antibody

☐ Small 100 µl
(10 Western mini-blots)

☐ Large 300 µl
(30 Western mini-blots)

Cell Signaling TECHNOLOGY®

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Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H, M, R, Mk	289 kDa	Rabbit

Background: The mammalian target of rapamycin, mTOR, also know as FRAP or RAFT (1-3), is a Ser/Thr protein kinase. mTOR acts as a sensor for ATP and amino acids, balancing the availability of nutrients and cell growth (4,5). When sufficient nutrients are available, mTOR responds to a phosphatidic acid-mediated signal (6), transmits a positive signal to p70 S6 kinase and participates in the inactivation of the eIF4E inhibitor, 4E-BP1. These events result in the translation of specific mRNA subpopulations. mTOR is phosphorylated at Ser2448 via the PI3 kinase/Akt signaling pathway and autophosphorylated at Ser2481 (7,8). mTOR plays a key role in cellular growth and homeostasis and its regulation is frequently altered in tumors. For these reasons, mTOR is currently under investigation as a potential target for anti-cancer therapy (9).

Specificity/Sensitivity: Phospho-mTOR (Ser2448) Antibody detects endogenous levels of mTOR only when phosphorylated at Ser2448.

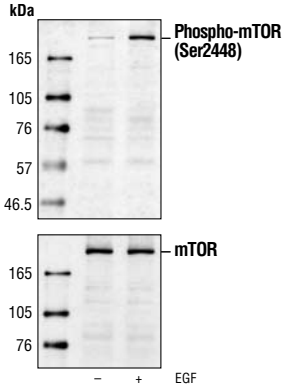
Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser2448 of human mTOR. Antibodies are purified by protein A and peptide affinity chromatography.

Selected Application References:

Bolster, D. R. et al. (2002) AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J. Biol. Chem.* 277, 23977–23980. Application: W.

Suzuki, A. et al. (2004) ARK5 is a tumor invasion-associated factor downstream of Akt signaling. *Mol. Cell. Biol.* 24, 3526–3535. Application: W.

Prieto, A.L. et al. (2007) Localization and signaling of the receptor protein tyrosine kinase Tyro3 in cortical and hippocampal neurons. *Neuroscience* 150, 319–34. Application: W.



Western blot analysis of extracts from 293 cells (starved for 16 hours) untreated or EGF-treated (100 ng/ml), using Phospho-mTOR (Ser2448) Antibody (upper) or control mTOR Antibody #2972 (lower).

Background References:

(1) Sabers, C.J. et al. (1995) *J. Biol. Chem.* 270, 815–822.

(2) Brown, E.J. et al. (1994) *Nature* 369, 756–758.

(3) Sabatini, D.M. et al. (1994) *Cell* 78, 35–43.

(4) Gingras, A.C. et al. (2001) *Genes Dev.* 15, 807–826.

(5) Dennis, P.B. et al. (2001) *Science* 294, 1102–1105.

(6) Fang, Y. et al. (2001) *Science* 294, 1942–1945.

(7) Navé, B.T. et al. (1999) *Biochem. J.* 344 Pt 2, 427–431.

(8) Peterson, R.T. et al. (2000) *J. Biol. Chem.* 275, 7416–7423.

(9) Huang, S. and Houghton, P.J. (2003) *Curr. Opin. Pharmacol.* 3, 371–377.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by Western blot.**

Recommended Antibody Dilutions:

Western Blotting	1:1000

Companion Products:

Phospho-eIF4G (Ser1108) Antibody #2441

mTOR Antibody #2972

Phospho-p70 S6 Kinase (Thr389) Antibody #9205

4E-BP1 Antibody #9452

Phospho-4E-BP1 (Thr70) Antibody #9455

Phospho-4E-BP1 (Thr37/46) Antibody #9459

Rapamycin (FRAP/mTOR Inhibitor) #9904

Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

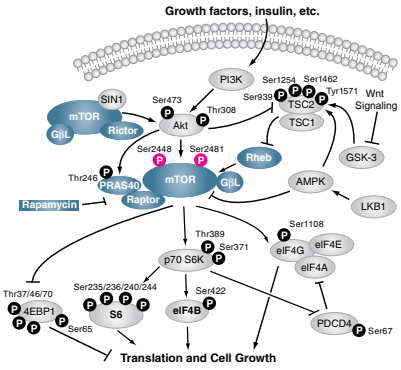
Anti-rabbit IgG, HRP-linked Antibody #7074

Prestained Protein Marker, Broad Range (Premixed Format) #7720

Biotinylated Protein Ladder Detection Pack #7727

20X LumiGLO® Reagent and 20X Peroxide #7003

Phospho-mTOR (Ser2481) Antibody #2974



IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.