

# mTOR Regulation Antibody Sampler Kit

✓ 1 Kit  
(6 x 40 µl)

**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com  
**Web** ■ www.cellsignal.com

rev. 07/15/09

This product is for *in vitro* research use only and is not intended for use in humans or animals. This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-mTOR (Ser2448) Antibody	2971	40 µl	289 kDa	Rabbit IgG
mTOR (7C10) Rabbit mAb	2983	40 µl	289 kDa	Rabbit IgG
Phospho-Raptor (Ser792) Antibody	2083	40 µl	150 kDa	Rabbit IgG
RagC Antibody	3360	40 µl	50 kDa	Rabbit IgG
Phospho-PRAS40 (Thr246) (C77D7) Rabbit mAb	2997	40 µl	40 kDa	Rabbit IgG
PRAS40 (D23C7) XP™ Rabbit mAb	2691	40 µl	40 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The mTOR Regulation Sampler Kit provides an economical means to evaluate the regulation of mTOR signaling by such proteins as phosphorylated Raptor, RagC and PRAS40. The kit contains enough primary and secondary antibodies to perform four western mini-blot experiments per primary antibody.

**Background:** The mammalian target of rapamycin (mTOR, FRAP, RAFT) is a Ser/Thr protein kinase (1-3) that functions as an ATP and amino acid sensor to balance nutrient availability and cell growth (4,5). When sufficient nutrients are available, mTOR responds to a phosphatidic acid-mediated signal to transmit a positive signal to p70 S6 kinase and participate in the inactivation of the eIF4E inhibitor, 4E-BP1 (6). 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 cell growth and homeostasis and may be abnormally regulated in tumors. For these reasons, mTOR is currently under investigation as a potential target for anti-cancer therapy (9).

The regulatory associated protein of mTOR (Raptor) was identified as an mTOR binding partner that mediates mTOR signaling to downstream targets (10,11). Raptor binds to mTOR substrates, including 4E-BP1 and p70 S6 kinase, through their TOR signaling (TOS) motifs and is required for mTOR-mediated phosphorylation of these substrates (12,13). PRAS40 interacts with raptor in insulin-deprived cells and inhibits the activation of the mTORC1 pathway. Phosphorylation of PRAS40 by Akt at Thr246 relieves PRAS40 inhibition of mTORC1 (14).

Recently raptor has been identified as a direct substrate of the AMP-activated protein kinase (AMPK) (15). AMPK phosphorylates raptor on Ser722/Ser792 (15). This phosphorylation is essential for inhibition of the raptor-containing mTOR complex 1 (mTORC1) and induces cell cycle arrest

when cells are stressed for energy (15). These findings suggest that raptor is a critical switch that correlates cell cycle progression with energy status.

The activity of mTORC1 kinase complex is modulated by energy levels, growth factors and amino acids (16,17). Recent studies found that RagA, RagB, RagC and RagD, the four related GTPases, interact with raptor in the mTORC1 complex (18,19). These interactions are both necessary and sufficient for mTORC1 activation in response to amino acid signals (18,19).

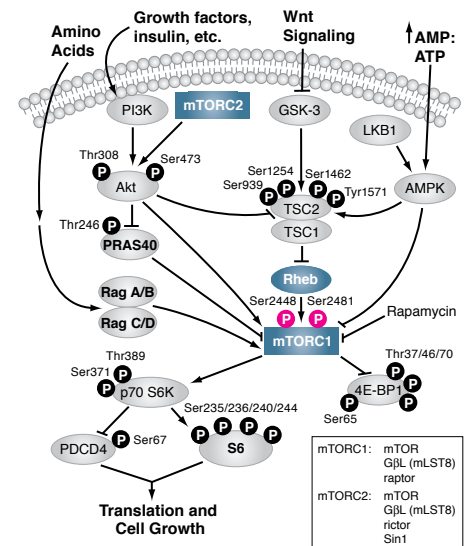
**Specificity/Sensitivity:** Each antibody in the mTOR Regulation Antibody Sampler Kit detects endogenous levels of its target protein. Activation state antibodies detect only target proteins phosphorylated at indicated residues. Phospho-Raptor (Ser792) Antibody may also detect non-specific signals of various molecular weights.

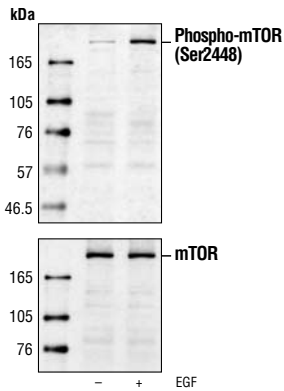
**Source/Purification:** Phospho-specific polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides (KLH-coupled) corresponding to residues surrounding Ser2448 of human mTOR and Ser792 of human raptor. RagC antibody is produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to the sequence of human RagC protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Phospho-PRAS40 (Thr246) (C77D7) Rabbit mAb is produced by immunizing animals with a synthetic phosphopeptide (KLH-coupled) derived from the sequence surrounding Thr246 of human PRAS40. Total protein monoclonal antibodies are produced by immunizing animals with synthetic peptides (KLH-coupled) corresponding to residues surrounding Ser2481 of human mTOR and the sequence of human PRAS40.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

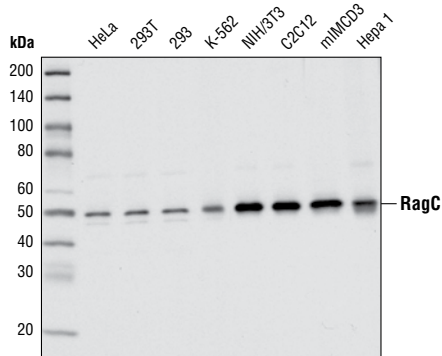
**Recommended Antibody Dilutions:**  
Western blotting 1:1000

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

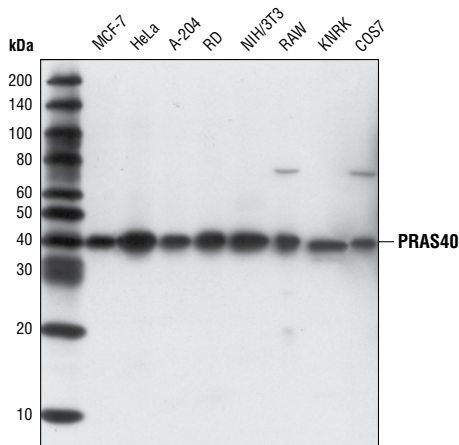




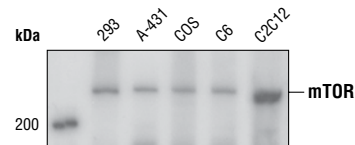
Western blot analysis of extracts from 293 cells (starved for 16 hours), untreated or EGF-treated (100 ng/ml), using **Phospho-mTOR (Ser2448) Antibody #2971** (upper) or control mTOR Antibody #2972 (lower).



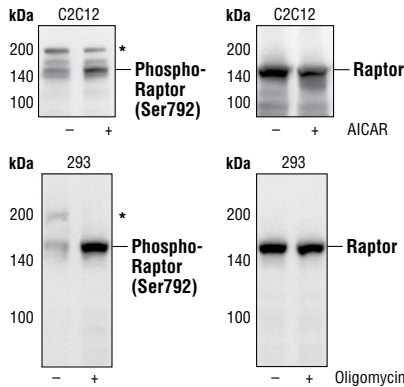
Western blot analysis of extracts from various cell types using **RagC Antibody #3360**.



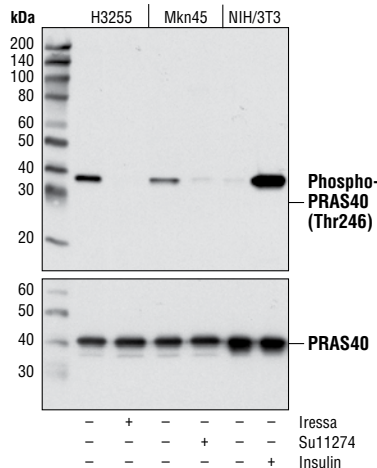
Western blot analysis of extracts from various cell types using **PRAS40 (D23C7) XP™ Rabbit mAb #2691**.



Western blot analysis of extracts from various cell types using **mTOR (7C10) Rabbit mAb #2983**.



Western blot analysis of C2C12 or 293 cells, untreated or treated with AICAR (0.5 mM for 30 minutes) or oligomycin (0.5  $\mu$ M for 30 minutes), using **Phospho-Raptor (Ser792) Antibody #2083** (upper and lower left) or Raptor Antibody #2280 (upper and lower right).  
\*Cross-reacting bands at 200 kDa.



Western blot analysis of extracts from serum starved H3255, Mkn45 and NIH/3T3 cells, untreated or treated with either Iressa (1  $\mu$ M, 3 hours), Su11274 (1  $\mu$ M, 3 hours) or insulin (150 nM, 15 minutes), using **Phospho-PRAS40 (Thr246) (C77D7) Rabbit mAb #2997** (upper) or PRAS40 (D23C7) Rabbit mAb #2691 (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.
- (10) Hara, K. et al. (2002) *Cell* 110, 177–89.
- (11) Kim, D.H. et al. (2002) *Cell* 110, 163–75.
- (12) Beugnet, A. et al. (2003) *J Biol Chem* 278, 40717–22.
- (13) Nojima, H. et al. (2003) *J Biol Chem* 278, 15461–4.
- (14) Vander Haar, E. et al. (2007) *Nat Cell Biol* 9, 316–23.
- (15) Gwinn, D.M. et al. (2008) *Mol Cell* 30, 214–26.
- (16) Hay, N. and Sonenberg, N. (2004) *Genes Dev* 18, 1926–45.
- (17) Wullschlegel, S. et al. (2006) *Cell* 124, 471–84.
- (18) Sancak, Y. et al. (2008) *Science* 320, 1496–501.
- (19) Kim, E. et al. (2008) *Nat Cell Biol* 10, 935–45.

## Western Immunoblotting Protocol (Primary Antibody 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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- 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%).
- Phototope<sup>®</sup>-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<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

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

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- 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<sup>®</sup> incubation and declines over the following 2 hours.