Store at -20°C

#9864

mTOR Regulation Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

Cell Signaling TECHNOLOGY®

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

rev. 06/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-mTOR (Ser2448) (D9C2) XP® Rabbit mAb	5536	20 µl	289 kDa	Rabbit IgG
mTOR (7C10) Rabbit mAb	2983	20 µl	289 kDa	Rabbit IgG
Phospho-Raptor (Ser792) Antibody	2083	20 µl	150 kDa	Rabbit IgG
RagC (D31G9) XP® Rabbit mAb	5466	20 µl	50 kDa	Rabbit IgG
Phospho-PRAS40 (Thr246) (C77D7) Rabbit mAb	2997	20 µl	40 kDa	Rabbit IgG
PRAS40 (D23C7) XP® Rabbit mAb	2691	20 µl	40 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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 for validation data and a complete listing of recommended companion products.

See 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 includes enough antibody to perform two western blot experiments with each 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 antibody is produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser792 of human raptor. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Phospho-specific monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to the sequence surrounding Thr246 of human PRAS40 and Ser2448 of human mTOR. Total protein monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Ser2481 of human mTOR, the sequence of human PRAS40, and the residues near the aminoterminus of human RagC.

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) Wullschleger, 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.

U.S. Patent No. 5,675,063

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#9864

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.