Store at -20C

#9964

# mTOR Pathway Antibody Sampler Kit

☑ 1 Kit
(6 X 20 µl)

Cell Signaling TECHNOLOGY®

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

| Products Included | Product # | Quantity | Mol. Wt. | Source |
|---|---|---|---|---|
| Phospho-mTOR (Ser2448) (D9C2) XP® Rabbit mAb | 5536 | 20 µl | 289 kDa | Rabbit IgG |
| Phospho-mTOR (Ser2481) Antibody | 2974 | 20 µl | 289 kDa | Rabbit IgG |
| mTOR (7C10) Rabbit mAb | 2983 | 20 µl | 289 kDa | Rabbit IgG |
| Raptor (24C12) Rabbit mAb | 2280 | 20 µl | 150 kDa | Rabbit IgG |
| Rictor (53A2) Rabbit mAb | 2114 | 20 µl | 200 kDa | Rabbit IgG |
| GβL (86B8) Rabbit mAb | 3274 | 20 µl | 37 kDa | Rabbit IgG |
| Anti-rabbit IgG, HRP-linked Antibody | 7074 | 100 µl | | Goat |

**See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.**

**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 antibodies.*

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

**Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.**

**Description:** The mTOR Pathway Antibody Sampler Kit contains reagents to investigate the control of protein translation, cell growth, and proliferation through mTOR signaling within cells. 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) interacts with mTOR to mediate mTOR signaling to downstream targets (10,11). Raptor binds to mTOR substrates, such as 4E-BP1 and p70 S6 kinase, through their TOR signaling (TOS) motifs and is required for mTOR-mediated substrate phosphorylation (12,13). Binding of the FKBP12-rapamycin complex to mTOR inhibits mTOR-raptor interaction, which suggests a mechanism for the inhibition of mTOR signaling by rapamycin (14). This mTOR-raptor interaction and its regulation by nutrients and/or rapamycin is dependent on a protein called GβL (15). GβL is part of the rapamycin-insensitive complex between mTOR and rictor (rapamycin-insensitive companion of mTOR) and may mediate rictor-mTOR signaling to PKCα and other downstream targets (16). The rictor-mTOR complex has been identified as the previously elusive PDK2 responsible for the phosphorylation of Akt/PKB at Ser473, which is required for PDK1 phosphorylation of Akt/PKB at Thr308 and full activation of Akt/PKB (17).

**Specificity/Sensitivity:** Each total antibody in the mTOR Pathway Sampler Kit recognizes only its specific target. Each phospho-specific antibody detects the intended target only when phosphorylated at the indicated site.

**Source/Purification:** Polyclonal antibody is produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser2481 of human mTOR. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser2448 of human mTOR, Gln1681 of human Rictor, Gln210 of human GβL and human Raptor.

**Background References:**

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(4) Gingras, A.C. et al. (2001) *Genes Dev.* 15, 807–826.
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(7) Navé, B.T. et al. (1999) *Biochem. J.* 344 Pt 2, 427–431.
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(10) Hara, K. et al. (2002) *Cell* 110, 177–189.
(11) Kim, D.H. et al. (2002) *Cell* 110, 163–175.
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(13) Nojima, H. et al. (2003) *J. Biol. Chem.* 278, 15461–15464.
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(15) Kim, D.H. et al. (2003) *Mol. Cell* 11, 895–904.
(16) Sarbassov, D.D. et al. (2004) *Curr. Biol.* 14, 1296–1302.
(17) Sarbassov, D.D. et al. (2005) *Science* 307, 1098–1101.

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**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.

# 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.