### mTOR Regulation Antibody Sampler Kit

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

<table>
<thead>
<tr>
<th>Products Included</th>
<th>Product #</th>
<th>Quantity</th>
<th>Mol. Wt.</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospho-mTOR (Ser2448) (D9C2) XP® Rabbit mAb</td>
<td>5536</td>
<td>20 µl</td>
<td>289 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>mTOR (7C10) Rabbit mAb</td>
<td>2983</td>
<td>20 µl</td>
<td>289 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Phospho-Raptor (Ser792) Antibody</td>
<td>2083</td>
<td>20 µl</td>
<td>150 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>RagC (D3169) XP® Rabbit mAb</td>
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<td>20 µl</td>
<td>50 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Phospho-PRAS40 (Thr246) (C77D7) Rabbit mAb</td>
<td>2997</td>
<td>20 µl</td>
<td>40 kDa</td>
<td>Rabbit IgG</td>
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<tr>
<td>PRAS40 (D23C7) XP® Rabbit mAb</td>
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<td>20 µl</td>
<td>40 kDa</td>
<td>Rabbit IgG</td>
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<tr>
<td>Anti-rabbit IgG, HRP-linked Antibody</td>
<td>7074</td>
<td>100 µl</td>
<td></td>
<td>Goat</td>
</tr>
</tbody>
</table>

**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 eff4E 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).

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 Ser72/792 (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 Ser2481 of human raptor. The sequence surrounding Thr246 of human PRAS40 and the sequence surrounding Ser2481 of human mTOR, the sequence of human PRAS40, and the sequence of human RagC are used as immunogens. Specific monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser2481 of human mTOR, the sequence of human PRAS40, and the sequence of human RagC.

**Background References:**

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 dH2O, mix.
2. 10X Tris Buffered Saline (TBS): (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH2O, 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 3X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH2O.
4. 10X Tris-Glycine SDS Running Buffer: (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH2O, 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 dH2O, mix.
6. 10X Tris Buffered Saline with Tween® 20 (TBST): (#9997) To prepare 1 L 1X TBST: add 100 ml 1X TBST to 900 ml dH2O, 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.
13. Prestained Protein Marker, Broad Range (Premixed Format): (#7720)
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 acrate 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 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 cm²) 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.