



## Anti-TOR Signaling Sampler Set

**CATALOG No: 54306**

### **BACKGROUND:**

The mammalian Target of Rapamycin (TOR, also known as mTOR) is an evolutionarily conserved serine/threonine kinase that regulates cell growth and cell cycle progression through its ability to integrate signals from nutrient levels and growth factors (reviewed in 1). TOR regulation is accomplished through a network of various activators and repressors. It is phosphorylated by Akt, whose activity is indirectly inhibited by the lipid phosphatase PTEN (2). TOR is normally associated with the regulatory proteins RAPTOR, a scaffold protein whose binding by TOR substrates is necessary for effective TOR-catalyzed phosphorylation (3), and GβL, which stimulates TOR's kinase activity towards downstream proteins (4). It is further regulated by the proteins Rheb, TSC1 and TSC2, which act to modulate TOR activity (5, 6). The downstream targets of TOR are thought to be the ribosomal protein S6 kinases and the eukaryotic initiation factor 4E binding proteins (4EBPs) whose activation leads to increased protein translation and cell growth (7).

### **KIT CONTENTS:**

Anti-TOR (NT), (25 µg).  
Anti-Raptor (IN), (25 µg).  
Anti-GβL (CT), (25 µg).  
Anti-Rheb (IN), (25 µg).  
Anti-TSC1 (CT), (25 µg).  
Anti-TSC2 (CT), (25 µg).  
Anti-4E-BP1, (25 µg).  
Anti-Akt1, **Catalog No. 54219 (25 µg)**.

### **SOURCE AND REACTIVITY:**

Rabbit polyclonal antibodies were raised against peptide sequences corresponding to each of the target proteins.

### **APPLICATION:**

The following concentration ranges are recommended starting points for this product.

**WB:** 1- 4 µg/ml

**IHC**

### **RELATED PRODUCTS:**

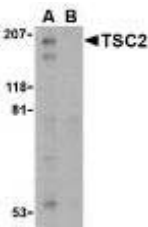
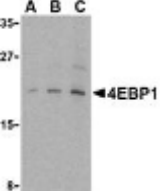
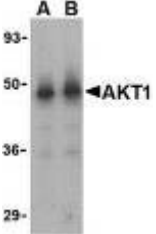

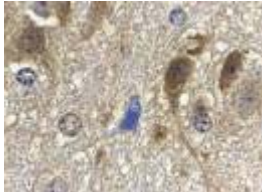
Mouse Brain Tissue Lysate (for Rheb), **Catalog No. 29507**  
3T3 Whole Cell Lysate (for 4E-BP1), **Catalog No. 29515**

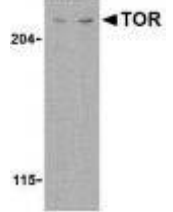
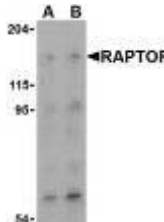
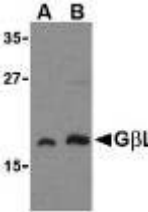
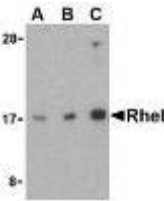
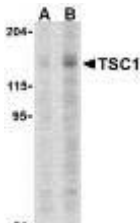
### **STORAGE:**

The antibodies are supplied as affinity chromatography purified IgG, in 1X PBS containing 0.02% Sodium Azide. Store at 2-8 °C for up to 1 year. Avoid repeated freeze thaw cycles.

### **REFERENCES:**

1. Shamji, AF. et al. *Mol. Cell* **12**, 271 (2003).
2. Nave, BT. et al. *Biochem. J.* **344**, 427 (1999).
3. Yonezawa, K. et al. *Biochem. Biophys. Res. Commun.* **313**, 437 (2004).
4. Fingar, DC. et al. *Oncogene* **23**, 3151 (2004).
5. Inoki, K. et al. *Genes Dev.* **17**, 1829 (2003).
6. Tee, AR. et al. *Proc. Natl. Acad. Sci. USA* **99**, 13571 (2002).
7. Fingar, DC. et al. *Oncogene* **23**, 3151 (2004).

	<p>Western blot analysis of TSC2 in L1210 cell lysate with anti-TSC2 (3507) at 1 µg/ml in the (A) absence and (B) presence of blocking peptide.</p>
	<p>anti-4E-BP1</p>
	<p>Western blot analysis of Akt1 in human liver cell lysate with anti-Akt1 (3519) at (A) 1 and (B) 2 µg/ml.</p>
	<p>Immunohistochemical staining of L1210 cells using TOR antibody (3585) at 2 µg/ml.</p>
	<p>Immunohistochemistry of GβL in mouse brain tissue with GβL antibody at 10 µg/ml.</p>

	<p>Western blot analysis of TOR in L1210 cell lysate with anti-TOR (3485) at (A) 1 and (B) 2 µg/ml.</p>
	<p>Western blot analysis of Raptor in L1210 cell lysate with anti-Raptor (3493) at (A) 2 and (B) 4 µg/ml.</p>
	<p>Western blot analysis of GβL in human brain cell lysate with anti-GβL (3495) at (A) 1 and (B) 2 µg/ml.</p>
	<p>Western blot analysis of Rheb in mouse brain cell lysate with anti-Rheb (3501) at (A) 1, (B) 2, and (C) 4 µg/ml.</p>
	<p>Western blot analysis of TSC1 in C2C12 cell lysate with anti-TSC1 (3503) at (A) 2 and (B) 4 µg.</p>

**WESTERN BLOT PROTOCOL:**

- 1.) Load 20-25 µg of whole cell lysate per lane in an SDS-PAGE mini gel.
- 2.) Run at 20 mA per gel until the dye front is close to the bottom.
- 3.) Transfer the proteins to a nitrocellulose membrane at 250 mA in transfer buffer for 1-4 hr, depending on the size of the target protein.
- 4.) Incubate the blot with blocking buffer (5% non-fat dry milk in TBS) overnight at 4°C or 2 hr at room temperature (RT).
- 5.) Incubate the blot with primary antibody (diluted 1:250 to 1:1000 in blocking buffer) for 1 hr in blocking buffer at RT.
- 6.) Wash the blot 3 x 10 min in washing buffer (TBS containing 0.1% Tween 20) with shaking.
- 7.) Incubate blot with anti-rabbit IgG-HRP conjugate (diluted 1:10,000 -1:2,000 in blocking buffer) for 1 hr in blocking buffer at RT.
- 8.) Wash 3 x 10 min in washing buffer with shaking.
- 9.) Drain washing buffer, add ECL solution and develop for 1 min.
- 10.) Expose to X-ray film for 1 to 30 min.

**MATERIALS NEEDED:**

- Nitrocellulose membrane
- Non-fat dry milk
- Tween-20
- Antibody detection kit

## TBS:

- 125 mM NaCl
- 25 mM Tris pH 8.0
- 0.1% Tween 20

## SDS/Running Buffer:

- 25 mM Tris
- 192 mM Glycine
- 0.1% SDS

## Transfer Buffer:

- 20 mM Tris
- 150 mM Glycine
- 20% methanol
- 0.038% SDS