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# Polyclonal Antibody to 40S ribosomal protein S6 (RPS6) pSer244/247 - Aff - Purified

**Catalog No.:** BP7084

**Quantity:** 0.1 ml

**Background:** 40S ribosomal protein S6 (also known as RPS6) is a ~31 kDa substrate of p70 S6 kinase (p70S6K) and a major component of translational machinery involved in protein synthesis, cell growth, proliferation, and metabolism. Phosphorylation of RPS6 is rapamycin and wortmannin-sensitive as its activation is mediated by mTOR and PI3K pathways. Ribosomal protein S6 undergoes phosphorylation on multiple serines in the carboxyl terminal region in the order 236->235->240->244->247, due to the positions of these amino acid residues on the α-helix. Hyperphosphorylation of ribosomal protein S6 stimulates protein synthesis that mediates progression through the cell cycle.

**Host:** Rabbit

**Immunogen:** Chemically synthesized phosphopeptide derived from the region of human RPS6 that contains serines 244 and 247.
**Remarks:** The sequence is conserved in mouse and rat.

**Format:** **State:** Liquid Ig fraction
**Purification:** Sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated RPS6. The final product is generated by affinity chromatography using a RPS6-derived peptide that is phosphorylated at serines 244 and 247.
**Buffer System:** Dulbecco's phosphate buffered saline (without Mg2+ and Ca2+), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier and 0.05 % sodium azide as preservative

**Applications:** Western blot (1:1000).
Positive control: HeLa +/- TNF-α or anisomycin.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

**Specificity:** This antibody detects RPS6. Does not cross react with RPS6 phosphorylated on serines 235 and 236.
**Species:** Human, mouse, rat.
Other species not tested.

**Storage:** Store at 2 - 8 °C up to one week or (in aliquots) at -20 °C for longer. Centrifuge vial before opening. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

**General Readings:**

Pende, M., et al. (2004) S6K1(-/-)/S6K2(-/-) mice exhibit perinatal lethality and rapamycin-sensitive 5'-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. Mol. Cell. Biol. 24(8):3112-3124.
Mourani, P.M., et al. (2004) Unique, highly proliferative growth phenotype expressed by embryonic and neointimal smooth muscle cells is driven by constitutive Akt, mTOR, and p70S6K signaling and is actively repressed by PTEN. Circulation 109(10):1299-1306.
Lekmine, F., et al. (2004) Interferon-gamma engages the p70 S6 kinase to regulate phosphorylation of the 40S S6 ribosomal protein. Exp. Cell. Res. 295(1):173-182.
Tuhackova, Z., et al. (2004) IL2-dependent phosphorylation of 40S ribosomal protein S6 is controlled by PI-3K/mTOR signalling in CTLL2 cells. Int. J. Mol. Med. 13(4):601-605.
Ly, C., et al. (2003) Bcr-Abl kinase modulates the translation regulators ribosomal protein S6 and 4E-BP1 in chronic myelogenous leukemia cells via the mammalian target of rapamycin. Cancer Res. 63(18):5716-5722.
Shah, O.J., et al. (2003) Mitotic regulation of ribosomal S6 kinase 1 involves Ser/Thr, Pro phosphorylation of consensus and non-consensus sites by Cdc2. J. Biol. Chem. 278(18):16433-16442.
Stewart, M.J. and G. Thomas (1994) Mitogenesis and protein synthesis: a role for ribosomal protein S6 phosphorylation? Bioessays. 16(11):809-815. Review. Ferrari, S., et al. (1991) Mitogen-activated 70K S6 kinase. Identification of in vitro 40S ribosomal S6 phosphorylation sites. J. Biol. Chem. 266(33):22770-22775.

**Protocols:**

**Western Blotting Procedure**

1. Lyse approximately 10e7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifugedat 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4°C.
12. Incubate the blocked blot with primary antibody at a 1:1000 dilution in Tris buffered saline supplemented with 3% BSA and 0.1% Tween 20 for one hour at room temperature or overnight at 4°C.

13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab)2 anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab)2 anti-rabbit IgG horseradish peroxidase conjugate in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO)
or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add 1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm BSA
0.1 mL Tween 20

Pictures:

**Peptide Competition** Lysates prepared from HeLa cells left untreated (1) or treated with TNF-α (2-5) were resolved by SDS-PAGE on a 14% polyacrylamide gel and transferred to PVDF. Membranes were blocked with a 5% BSA-TBST buffer for one hour at room temperature, and incubated with ribosomal protein S6 [pSpS244/247] antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2), the non-phosphopeptide corresponding to the immunogen (3), a generic phosphoserine-containing peptide (4), or, the phosphopeptide immunogen (5). After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG HRP conjugate and bands were detected using the Pierce SuperSignalTM method. The data show that only the peptide corresponding to ribosomal protein S6 [pSpS244/247] blocks the signal, verifying the specificity of the antibody.