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Product Information

Anti-phospho-S6-Kinase (pThr^{389})
produced in rabbit, affinity isolated antibody

Catalog Number **S6311**

Synonym: Anti-phospho-p70^{S6K} (pThr^{389})

Product Description

Anti-phospho-S6-Kinase (pThr^{389}) is produced in rabbit, using as immunogen a synthetic phospho-Thr^{389} peptide corresponding to residues around Thr^{389} of human p70 S6 kinase, conjugated to KLH,. This antibody is affinity-purified using protein A and peptide affinity chromatography.

Anti-phospho-S6-Kinase (pThr^{389}) detects p70 S6 kinase and p85 S6 kinase only when activated by phosphorylation at Thr^{389}. This antibody also recognizes some phosphorylated isoforms of PKC (Protein Kinase C) if present at high levels in cell extracts. Anti-phospho-S6-Kinase (pThr^{389}) reacts with human, rat and mouse p70 S6 kinase and may be used for immunoblotting.

p70 S6 kinase is a mitogen activated Ser/Thr protein kinase that is required for cell growth and G1 cell cycle progression.[1] p70 S6 kinase phosphorylates 40S subunit ribosomal protein S6 and is involved in translational control of 5'-oligopyrimidine tract mRNAs. [1] A second isoform, p85 S6 kinase, is derived from the same gene and is identical to p70 S6 kinase except for 23 extra residues at the N-terminus that encode a nuclear localizing signal. [1] The activity of p70 S6 kinase is controlled by multiple phosphorylation events at sites located within the catalytic, linker and pseudosubstrate domains. [1] Thr^{229} in the catalytic domain and Thr^{389} in the linker domain are critical for kinase function. [1] However, phosphorylation at Thr^{389} most clearly correlates with p70 kinase activity *in vivo*.[2] Phosphorylation of this site is stimulated by growth factors such as insulin, EGF, FGF, serum and some G-protein coupled receptor ligands. Phosphorylation is completely blocked by wortmannin, LY294002 (PI3 Kinase Inhibitors) and rapamycin (FRAP/TOR inhibitor). [1,3,4]

Reagents

Supplied in 10 mM sodium HEPES, pH 7.5, containing 150 mM sodium chloride, 100 µg/ml bovine serum albumin and 50% glycerol.

Storage/Stability

Store –20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting (chemiluminescent): recommended working dilution is 1:1,000 using extracts from insulin treated HeLa, COS, or C6 cells. A 1:1,000 dilution is also recommended for NIH-3T3 cells treated with 20% serum.

For immunoblotting, incubate membrane with diluted antibody in 5% bovine serum albumin (BSA), 1X Tris buffered saline and 0.1% TWEEN 20 at 2-8 °C with gentle shaking, overnight.

Immunoblotting Note: To reduce basal levels of p70 S6 kinase (Thr389) phosphorylation, plate and culture the cells in low serum (0.5% FBS) medium for 2 days. Before inducing phosphorylation, incubate the cells in serum-free medium for 2 hours and then change to fresh serum-free medium immediately before treatment.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration.

References

1. Pullen, N., and Thomas, G., *FEBS Lett*., 410, 78-82 (1997).
2. Weng, Q.P., et al., *J. Biol. Chem*., 273, 16621-
3. Polakiewicz, R.D., et al., *J. Biol. Chem*., 273, 23534-23541 (1998).
4. Jeffries, H.B., et al., *EMBO* J., 16, 3693-3704 (1997).

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