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ProductInformation

Anti-phospho-S6-kinase ($p70^{S6K}$)
(phosphothreonine 421/phosphoserine 424)
Developed in Rabbit,
Affinity Isolated Antibody

Product Number **S 6436**

Product Description

Anti-phospho-S6-kinase ($p70^{S6K}$) (phosphothreonine 421/phosphoserine424) is developed in rabbit**,** using a synthetic double phospho-Thr421/Ser424 peptide corresponding to residues around Thr421/Ser424 of human p70 S6 kinase and conjugated to KLH, as immunogen. This antibody is affinity-purified using protein A and peptide affinity chromatography.

Anti-phospho-S6-kinase ($p70^{S6K}$) (phosphothreonine 421/phosphoserine424) detects p70 S6 kinase and p85 S6 kinase only when activated by phosphorylation at Thr421/Ser424 and does not cross-react with other phosphoylated protein kinases. Anti-phospho-S6-kinase ($p70^{S6K}$) (phosphothreonine 421/phosphoserine424) reacts with human, rat and mouse p70 S6 kinase and may be used for immunoblotting and immuno-precipitation.

Anti-phospho-S6-kinase ($p70^{S6K}$) (phosphothreonine 421/phosphoserine424) is a mitogen activated Ser/Thr protein kinase that is required for cell growth and G1 cell cycle progression.[1-7] 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.[2, 3] Both isoforms lie on a mitogen activated signaling pathway that is distinct from the ras/MAP kinase cascade. The activity of p70 S6 kinase is controlled by multiple phosphorylation events at sites located within the catalytic and pseudosubstrate domains. Ser411, Thr421 and Ser424 lie within a Ser-Pro rich region located within the pseudosubstrate region.[5, 6] Phosphorylation at these sites is thought to activate p70 S6 kinase via relief of pseudosubstrate repression[5, 6], although most evidence suggests that other events may be necessary. These clustered sites are phosphorylated in response to mitogens by Ser/Pro directed kinases. Phosphorylation at these sites stimulated by EGF, FGF, FBS and TPA is completely blocked by rapamycin (FRAP/TOR inhibitor), whereas wortmannin (PI3 Kinase Inhibitor) blocks all but TPA stimulated phosphorylation at Ser411, Thr421 and Ser424.

Reagents

Anti-phospho-S6-kinase ($p70^{S6K}$) (phosphothreonine 389) is supplied as an affinity-isolated antibody 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 at 0 °C to –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

Recommended working dilution is 1:1,000 for immunoblotting (chemiluminescent) using an extract from serum-treated NIH-3T3 cells. 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 (Thr421/Ser424) 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.

Recommended working dilution for immunoprecipitation is 1:250.

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

References

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