SIGMA

ProductInformation

ANTI-STE20/PAK SUBDOMAIN VI
Developed in Rabbit, Affinity Isolated Antibody

Product Number **S6558**

Product Description

Anti-STE20/PAK is developed in rabbit using a synthetic peptide (RDIKSDNILLSMEGD-C) based upon the subdomain VI region (residues 738-752) of *S. cerevisiae* STE20 conjugated to KLH as immunogen. The antibody is purified using affinity chromatography.

Anti-STE20/PAK reacts specifically with recombinant *S. cerevisiae* STE20 protein expressed in E. coli and rat brain PAK (65 kD) by immunoblotting. It may react with other proteins that may be homologs of PAK in rat tissue extracts. The antibody reacts with rabbit, rat and yeast STE20/PAK. Other species cross-reactivity is unknown.

Anti-STE20/PAK may be used for the detection of STE20/PAK by immunoprecipitation and immunoblotting.

The STE20p protein kinase, the founding member of the STE20/PAK 1 family of protein kinases, plays important roles in a number of cellular processes in the yeast *Saccharomyces cerevisiae*.[1] The STE20p kinase is post-translationally modified by phosphorylation in a cell cycle-dependent manner. This modification is maximal during S phase. STE20p was initially identified because of its role in yeast mating.[2] Subsequently, the STE20p kinase was found to be required for pseudohyphal growth in diploid *S. cerevisiae* cells[3] and for invasive growth in nutrient limited haploid cells.[4] In addition, the kinase appears to share an essential function with the related kinase Cla4p.
The yeast STE20 kinase mammalian homolog is p21-activated kinase (PAK). Many of the signaling pathways leading to the execution of cytoskeletal architecture,[5-7] stimulation of DNA synthesis,[8] cellular transformation,[9-12] and signaling to the nucleus[13-18] involve PAKs[5] which are direct effectors of Rac1 and Cdc42.[19-21] Binding of these GTPases to a conserved p21-binding domain (PBD, also known as CRIB for Cdc42/Rac1 interactive binding), stimulates their serine/threonine kinase activities by a mechanism involving autophosphorylation.[19, 22] The important roles that PAKs play as effectors of Cdc42/Rac1 signaling have been established from genetic and biochemical studies in yeast and mammalian cells. STE20, acts in concert with Cdc42 in the pheromone response to activate a MAP kinase cascade leading to transcription of genes required for cell cycle arrest. The same protein functions as an effector of Cdc42 to activate a different MAP kinase cascade leading to filamentous growth in response to nitrogen starvation.[23] The yeast model in which STE20/PAK links Cdc42 to transcriptional and cytoskeletal events is paralleled in mammalian cells. Constitutively activated PAK mutants can activate the c-Jun N-terminal kinase MAP kinase cascade leading to transcriptional control[16,17] and can mimic some, although not all, of the effects of Rac1 or Cdc42 on cytoskeletal organization.[22, 24]
The apparent multiplicity of PAK-mediated signaling pathways suggests that PAK activity must be tightly regulated. This has been made all the more clear from the observations that PAK1 function is required for cellular transformation by Ras[12] and that PAK2 activation is involved in Fas-mediated apoptosis.[25,26]

Reagents

The product is supplied as affinity isolated antibody in 0.07 mM tris-glycine buffer, pH 7.0, containing 30% glycerol.

Storage/Stability

Store at 0°C to −20°C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P3813).
2. Add 4 µg of Anti-STE20/PAK to 0.5 - 1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (Sigma Product No. P2545).

5. Gently rock reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer* or PBS.
7. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer, or the agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

*Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4, and 1 mM NaF.

Product Profile
Recommended working concentration is 0.5 - 2 µg/ml by immunoblotting using rabbit brain cytosol.

For immunoprecipitation, 4 µg will immunoprecipitate STE20 homologs (PAK) from 0.5 mg of a rabbit brain cytosol.

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

References

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