SANTA CRUZ BIOTECHNOLOGY, INC.

p-α/β/γPAK (Thr 402): sc-135684

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Three isoforms of serine/threonine kinases, designated αPAK p68, βPAK p65 and γPAK p62, have been shown to exhibit a high degree of sequence homology with the *S. cerevisiae* kinase Ste 20, involved in pheromone signaling. The α, β and γPAK isoforms complex specifically with Rac1 and Cdc42 in their active GTP-bound state, inhibiting their intrinsic GTPase activity leading to their autophosphorylation. There are eight sites of autophosphorylation on γPAK, including Ser 19, Ser 141 and Thr 402, and phosphorylation of Ser 141 and Thr 402 is correlated with γPAK activation. Once phosphorylated and their affinity for Rac/Cdc42 reduced, the PAK isoforms disassociate from the complex to seek downstream substrates. One such putative substrate is Mek kinase, an upstream effector of Mek4 which is involved in the JNK signaling pathway. While the PAK isoforms interact in a GTP-dependent manner with Rac1 and Cdc42, they do not interact with Rho.

REFERENCES

1. Didsbury, J., Weber, R.F., Bokoch, G.M., Evans, T. and Snyderman, R. 1989. Rac, a novel ras-related family of proteins that are *botulinum* toxic substrates. J. Biol. Chem. 264: 16378-16382.
2. Shinjo, K., Koland, J.G., Hart, M.J., Narasimhan, V., Johnson, D.I., Evans, T. and Cerione, R.A. 1990. Molecular cloning of the gene for the human placental GTP-binding protein G_p (G25K): identification of this GTP-binding protein as the human homolog of the yeast cell-division-cycle protein Cdc42. Proc. Natl. Acad. Sci. USA 98: 9853-9857.
3. Boguski, M.S. and McCormick, F. 1993. Proteins regulating Ras and its relatives. Nature 366: 643-654.
4. Lange-Carter, C.A., Pleiman, C.M., Gardner, A.M., Blumer, K.J. and Johnson, G.L. 1993. A divergence in the MAP kinase regulatory network defined by MEK kinase and Raf. Science 260: 315-319.
5. Manser, E., Leung, T., Salihuddin, H., Zhao, Z.S. and Lim, L. 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature 367: 40-46.
6. Yan, M., Dai, T., Deak, J.C., Kyriakis, J.M., Zon, L.I., Woodgett, J.R. and Templeton, D.J. 1994. Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1. Nature 372: 798-800.
7. Martin, G.A., Bollag, G., McCormick, F. and Abo, A. 1995. A novel serine kinase activated by racl/Cdc42Hs-dependent autophosphorylation is related to PAK65 and Ste20. EMBO J. 14: 1970-1978.
8. Yu, J.S., Chen, W.J., Ni, M.H., Chan, W.H. and Yang, S.D. 1998. Identification of the regulatory autophosphorylation site of autophosphorylation-dependent protein kinase (auto-kinase). Evidence that auto-kinase belongs to a member of the p21-activated kinase family. Biochem. J. 334: 121-131.

STORAGE

For immediate and continuous use, store at 4° C for up to one month. For sporadic use, freeze in working aliquots in order to avoid repeated freeze/thaw cycles. If turbidity is evident upon prolonged storage, clarify solution by centrifugation.

SOURCE

p-α/β/γPAK (Thr 402) is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Thr 402 of γPAK of rat origin.

PRODUCT

Each vial contains IgG in 100 µl of 10 mM HEPES with 150 mM NaCl, 50% glycerol and < 0.1% BSA.

APPLICATIONS

p-α/β/γPAK (Thr 402) is recommended for detection of Thr 402 phosphorylated γPAK and correspondingly phosphorylated αPAK and βPAK of mouse, rat and human origin by Western Blotting (starting dilution to be determined by researcher, dilution range 1:100-1:5000) and immunoprecipitation [1-2 µl per 100-500 µg of total protein (1 ml of cell lysate)].

p-α/β/γPAK (Thr 402) is also recommended for detection of Thr 402 phosphorylated γPAK and correspondingly phosphorylated αPAK and βPAK in additional species, including bovine and canine.

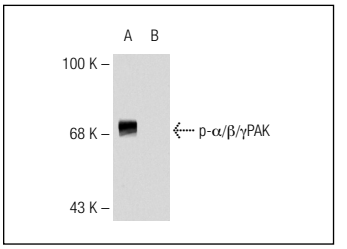
Molecular Weight of p-α/β/γPAK: 67/68/62 kDa.

Positive Controls: SK-N-MC+forskolin cell lysate: sc-2288, SK-N-MC cell lysate: sc-2237 or rat hippocampal tissue extract.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).

DATA



p-α/β/γPAK (Thr 402): sc-135684. Western blot analysis of α/β/γPAK phosphorylation in untreated (**A**) and lambda protein phosphatase (sc-200312A) treated (**B**) rat hippocampal tissue extracts.

RESEARCH USE

For research use only, not for use in diagnostic procedures.