SANTA CRUZ BIOTECHNOLOGY, INC.

αPAK (H-300): sc-11394

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., et al. 1989. Rac, a novel ras-related family of proteins that are botulinum toxic substrates. J. Biol. Chem. 264: 16378-16382.
2. Shinjo, K., et al. 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., et al. 1993. Proteins regulating Ras and its relatives. Nature 366: 643-654.
4. Manser, E., et al. 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature 367: 40-46.
5. Yan, M., et al. 1994. Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1. Nature 372: 798-800.

SOURCE

αPAK (H-300) is a rabbit polyclonal antibody raised against amino acids 246-470 mapping at the C-terminus of αPAK of human origin.

PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

APPLICATIONS

αPAK (H-300) is recommended for detection of αPAK, βPAK and γPAK of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

αPAK (H-300) is also recommended for detection of αPAK, βPAK and γPAK in additional species, including equine, canine, bovine, porcine and avian.

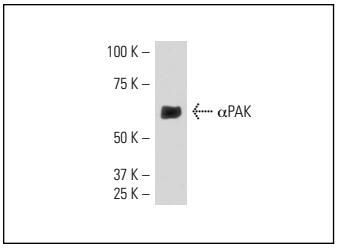
Molecular Weight of αPAK: 65 kDa.

Positive Controls: SJRH30 cell lysate: sc-2287, mouse brain extract: sc-2253 or rat brain extract: sc-2392.

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 A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

DATA



αPAK (H-300): sc-11394. Western blot analysis of αPAK expression in SJRH30 whole cell lysate.

SELECT PRODUCT CITATIONS

1. Loesberg, W.A., et al. 2008. Simulated microgravity activates MAPK pathways in fibroblasts cultured on microgrooved surface topography. Cell Motil. Cytoskeleton 65: 116-129.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.