SANTA CRUZ BIOTECHNOLOGY, INC.

p-αPAK (Ser 199/204): sc-33531

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The Power to Question

BACKGROUND

p21-activated kinases (PAK) are serine/threonine kinases that link Rho GTPases to cytoskeletal reorganization and nuclear signaling. Three common isoforms are αPAK p68, βPAK p65 and γPAK p62. α, β and γPAK isoforms associate with Rac 1 and Cdc42 in their active GTP-bound state, inhibiting their intrinsic GTPase activity and mediating their autophosphorylation. γPAK can undergo phosphorylation on Ser-19, Ser 141 and Thr 402, and phosphoryl-ation of Ser 141 and Thr 402 correlates with γPAK activation. Autophosphoryl-ation of αPAK Thr 423 (Thr 402 for βPAK and Thr 421 for γPAK) is catalyzed by Cdc42 and is required for kinase activation of PAK. Once phosphorylated and their affinity for Rac/Cdc42 reduced, PAK isoforms disassociate from the complex to seek downstream substrates. One such substrate is MEK kinase, an upstream effector of MEK4 which is involved in the JNK signaling pathway.

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 Rac 1. 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.
6. Minden, A., et al. 1994. Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. Science 266: 1719-1723.
7. Coso, O.A., et al. 1995. The small GTP-binding proteins Rac 1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell 81: 1137-1146.
8. Martin, G.A., et al. 1995. A novel serine kinase activated by Rac 1/Cdc42Hs-dependent autophosphorylation is related to PAK65 and STE20. EMBO J. 14: 1970-1978.

CHROMOSOMAL LOCATION

Genetic locus: PAK1 (human) mapping to 11q13.5; Pak1 (mouse) mapping to 7 E2.

SOURCE

p-αPAK (Ser 199/204) is a rabbit polyclonal antibody raised against a short amino acid sequence containing Ser 199 and Ser 204 dually phosphorylated αPAK of human origin.

STORAGE

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

PRODUCT

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

Blocking peptide available for competition studies, sc-33531 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS

p-αPAK (Ser 199/204) is recommended for detection of Ser 199 and Ser 204 phosphorylated αPAK 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); may cross-react with correspondingly phosphorylated βPAK and γPAK.

p-αPAK (Ser 199/204) is also recommended for detection of correspondingly phosphorylated αPAK in additional species, including equine, canine, bovine and porcine.

Suitable for use as control antibody for αPAK siRNA (h): sc-29700, αPAK siRNA (m): sc-29701, αPAK shRNA Plasmid (h): sc-29700-SH, αPAK shRNA Plasmid (m): sc-29701-SH, αPAK shRNA (h) Lentiviral Particles: sc-29700-V and αPAK shRNA (m) Lentiviral Particles: sc-29701-V.

Molecular Weight of p-αPAK: 65 kDa.

Positive Controls: SK-N-SH cell lysate: sc-2410.

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). 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.

SELECT PRODUCT CITATIONS

1. Sebastián-Serrano, A., et al. 2012. Pαx6 expression in postmitotic neurons mediates the growth of axons in response to SFRP1. PLoS ONE 7: e31590.

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.