

# p- $\alpha$ PAK (Thr 212): sc-101772

## BACKGROUND

Three isoforms of serine/threonine kinases, designated  $\alpha$ PAK p68,  $\beta$ PAK p65 and  $\gamma$ PAK p62, have been shown to exhibit a high degree of sequence homology with the *S. cerevisiae* kinase Ste 20, involved in pheromone signaling. The  $\alpha$ ,  $\beta$  and  $\gamma$ PAK isoforms complex specifically with Rac 1 and Cdc42 in their active GTP-bound state, inhibiting their intrinsic GTPase activity leading to their autophosphorylation. There are eight sites of autophosphorylation on  $\gamma$ PAK, including Ser 19, Ser 141 and Thr 402, and phosphorylation of Ser 141 and Thr 402 is correlated with  $\gamma$ PAK 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 MEK-4 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

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- 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.
- Boguski, M.S. and McCormick, F. 1993. Proteins regulating Ras and its relatives. *Nature* 366: 643-654.
- Manser, E., et al. 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac 1. *Nature* 367: 40-46.
- Yan, M., et al. 1994. Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1. *Nature* 372: 798-800.
- Minden, A., et al. 1994. Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. *Science* 266: 1719-1723.
- 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.
- 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 11q14.1; Pak1 (mouse) mapping to 7 E2.

## SOURCE

p- $\alpha$ PAK (Thr 212) is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Thr 212 of  $\alpha$ PAK of human origin.

## PRODUCT

Each vial contains 100  $\mu$ g IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

## APPLICATIONS

p- $\alpha$ PAK (Thr 212) is recommended for detection of Thr 212 phosphorylated  $\alpha$ PAK of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1–2  $\mu$ g per 100–500  $\mu$ g of total protein (1 ml of cell lysate)], immunofluorescence and immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500).

Suitable for use as control antibody for  $\alpha$ PAK siRNA (h): sc-29700 and  $\alpha$ PAK siRNA (m): sc-29701.

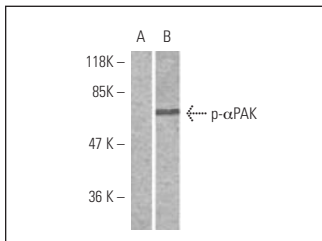
Molecular Weight of p- $\alpha$ PAK: 65 kDa.

Positive Controls: human breast carcinoma tissue or 293 whole cell lysate.

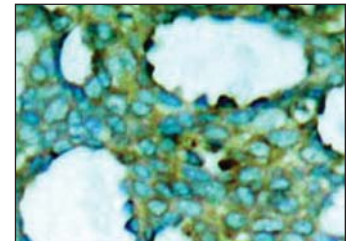
## RECOMMENDED SECONDARY REAGENTS

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) 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. 4) Immunohistochemistry: use ImmunoCruz™: sc-2051 or ABC: sc-2018 rabbit IgG Staining Systems.

## DATA



p- $\alpha$ PAK (Thr 212): sc-101772. Western blot analysis of phosphorylated  $\alpha$ PAK expression in untreated (A) and forskolin-treated (B) 293 whole cell lysates.



p- $\alpha$ PAK (Thr 212): sc-101772. Immunoperoxidase staining of formalin-fixed, paraffin-embedded human breast carcinoma tissue showing cytoplasmic staining.

## 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](http://www.scbt.com) or our catalog for detailed protocols and support products.