

$\alpha/\beta/\gamma$ PAK (D-8): sc-166174

The Power to Question

## 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 Ste20, involved in pheromone signaling. The  $\alpha$ ,  $\beta$  and  $\gamma$ PAK 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  $\gamma$ 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 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 Gp (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.

## SOURCE

$\alpha/\beta/\gamma$ PAK (D-8) is a mouse monoclonal antibody raised against amino acids 263-345 mapping within an internal region of  $\beta$ PAK of mouse origin.

## PRODUCT

Each vial contains 200  $\mu$ g IgG<sub>1</sub> kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

$\alpha/\beta/\gamma$ PAK (D-8) is available conjugated to agarose (sc-166174 AC), 500  $\mu$ g/0.25 ml agarose in 1 ml, for IP; to HRP (sc-166174 HRP), 200  $\mu$ g/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-166174 PE), fluorescein (sc-166174 FITC), Alexa Fluor<sup>®</sup> 488 (sc-166174 AF488), Alexa Fluor<sup>®</sup> 546 (sc-166174 AF546), Alexa Fluor<sup>®</sup> 594 (sc-166174 AF594) or Alexa Fluor<sup>®</sup> 647 (sc-166174 AF647), 200  $\mu$ g/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor<sup>®</sup> 680 (sc-166174 AF680) or Alexa Fluor<sup>®</sup> 790 (sc-166174 AF790), 200  $\mu$ g/ml, for Near-Infrared (NIR) WB, IF and FCM.

## APPLICATIONS

$\alpha/\beta/\gamma$ PAK (D-8) is recommended for detection of  $\alpha$ PAK,  $\beta$ PAK and  $\gamma$ PAK of mouse, rat and human origin by Western Blotting (starting dilution 1:100, 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 (starting dilution 1:50, dilution range 1:50-1:500), immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

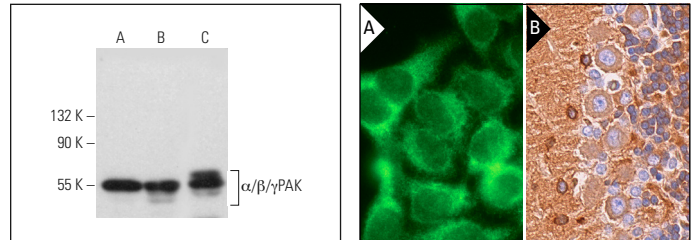
Molecular Weight of  $\alpha/\beta/\gamma$ PAK: 67/68/62 kDa.

Positive Controls: HeLa whole cell lysate: sc-2200, SH-SY5Y cell lysate: sc-3812 or EOC 20 whole cell lysate: sc-364187.

## RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 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 m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850. 4) Immunohistochemistry: use m-IgG $\kappa$  BP-HRP: sc-516102 with DAB, 50X: sc-24982 and Immunohistomount: sc-45086, or Organo/Limonene Mount: sc-45087.

## DATA



$\alpha/\beta/\gamma$ PAK (D-8): sc-166174. Western blot analysis of  $\alpha/\beta/\gamma$ PAK expression in HeLa (A), SH-SY5Y (B) and EOC 20 (C) whole cell lysates.

$\alpha/\beta/\gamma$ PAK (D-8): sc-166174. Immunofluorescence staining of methanol-fixed HeLa cells showing cytoplasmic localization (A). Immunoperoxidase staining of formalin fixed, paraffin-embedded rat cerebellum tissue showing cytoplasmic staining of Purkinje cells, cells in granular layer and cells in molecular layer (B).

## SELECT PRODUCT CITATIONS

1. Yuan, W., et al. 2016. MicroRNA-126 inhibits colon cancer cell proliferation and invasion by targeting the chemokine (C-X-C motif) receptor 4 and Ras homolog gene family, member A, signaling pathway. *Oncotarget* 7: 60230-60244.
2. Shah, K.N., et al. 2019. Aurora kinase A drives the evolution of resistance to third-generation EGFR inhibitors in lung cancer. *Nat. Med.* 25: 111-118.
3. Xiao, J., et al. 2019. lncRNA HOTAIR promotes gastric cancer proliferation and metastasis via targeting miR-126 to active CXCR4 and RhoA signaling pathway. *Cancer Med.* 8: 6768-6779.

## 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) for detailed protocols and support products.

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