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SANTA CRUZ BIOTECHNOLOGY, INC.

αPAK siRNA (m2): sc-270016

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.
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. Martin, G.A., et al. 1995. A novel serine kinase activated by Rac1/Cdc42Hs-dependent autophosphorylation is related to PAK65 and STE20. EMBO J. 14: 1970-1978.

CHROMOSOMAL LOCATION

Genetic locus: Pak1 (mouse) mapping to 7 E2.

PRODUCT

αPAK siRNA (m2) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see αPAK shRNA Plasmid (m2): sc-270016-SH and αPAK shRNA (m2) Lentiviral Particles: sc-270016-V as alternate gene silencing products.

For independent verification of αPAK (m2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270016A, sc-270016B and sc-270016C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

αPAK siRNA (m2) is recommended for the inhibition of αPAK expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

αPAK (A-6): sc-166887 is recommended as a control antibody for monitoring of αPAK gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor αPAK gene expression knockdown using RT-PCR Primer: αPAK (m2)-PR: sc-270016-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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

PROTOCOLS

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