SANTA CRUZ BIOTECHNOLOGY, INC.

αPIX siRNA (m): sc-39147

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The Power to Question

BACKGROUND

The serine/threonine kinase, p21 activated kinase (PAK), is a downstream effector of the small GTPases Cdc42 and Rac. PAK associates with NCK, the p85 and p110 subunits of PI 3-kinase, and PIX (PAK-interacting exchange factor) in a focal complex. The binding of PIX is necessary for the localization and activation of PAK in the Cdc42 to Rac signaling pathway, and this binding occurs through the high affinity of the N-terminal SH3 domain of PIX for a conserved proline rich PAK sequence. PIX exists as two isoforms, α and β and both are highly expressed in heart, muscle, and thymus tissues of human and rat. αPIX is phosphorylated via PDGF and EphB2 receptor signaling pathways or through association with PI 3-kinase. The αPIX isoform predominantly acts as a guanine nucleotide exchange factor (GEF) on Rac, which may mediate lamellipodia formation.

REFERENCES

1. Manser, E., et al. 1998. PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. Mol. Cell 1: 183-192.
2. Obermeier, A., et al. 1998. PAK promotes morphological changes by acting upstream of Rac. EMBO J. 17: 4328-4339.
3. Yoshii, S., et al. 1999. αPIX nucleotide exchange factor is activated by interaction with phosphatidylinositol 3-kinase. Oncogene 18: 5680-5690.
4. Daniels, R.H., et al. 1999. αPix stimulates p21-activated kinase activity through exchange factor-dependent and -independent mechanisms. J. Biol. Chem. 274: 6047-6050.
5. Turner, C.E., et al. 1999. Paxillin LD4 motif binds PAK and PIX through a novel 95 kDa ankyrin repeat, ARF-GAP protein: a role in cytoskeletal remodeling. J. Cell Biol. 145: 851-863.
7. Li, Z., et al. 2003. Directional sensing requires $G_{\beta\gamma}$-mediated PAK1 and PIX α-dependent activation of Cdc42. Cell 114: 215-227.
8. Feng, Q., et al. 2004. Novel regulatory mechanisms for the Dbl family guanine nucleotide exchange factor Cool-2/α-Pix. EMBO J. 23: 3492-3504.
9. Baird, D., et al. 2005. The Cool-2/α-Pix protein mediates a Cdc42-Rac signaling cascade. Curr. Biol. 15: 1-10.

CHROMOSOMAL LOCATION

Genetic locus: Arhgef6 (mouse) mapping to X A5.

PRODUCT

αPIX siRNA (m) 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 αPIX shRNA Plasmid (m): sc-39147-SH and αPIX shRNA (m) Lentiviral Particles: sc-39147-V as alternate gene silencing products.

For independent verification of αPIX (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-39147A, sc-39147B and sc-39147C.

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

αPIX siRNA (m) is recommended for the inhibition of αPIX 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor αPIX gene expression knockdown using RT-PCR Primer: αPIX (m)-PR: sc-39147-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.