

# ACK siRNA (m): sc-29633

## BACKGROUND

The Ras-related Rho subfamily of GTP-binding proteins (p21s), which includes Rho, Rac and Cdc42Hs, is implicated in different aspects of cytoskeletal organization. These proteins resemble Ras p21 in that their active GTP-bound form is inactivated by intrinsic hydrolysis of the GTP to GDP, which can be stimulated by GTPase-activating proteins (GAPs). ACK, a tyrosine kinase that specifically binds Cdc42Hs in its GTP-bound form, has been described. This binding is mediated by a unique sequence of 47 amino acids C-terminal to an SH3 domain and inhibits both the intrinsic and GAP-stimulated GTPase activity of Cdc42Hs. These findings suggest that ACK may represent a new class of proteins that sustains the GTP-bound active form of the Rho subfamily of GTP binding proteins and which is directly linked to a tyrosine phosphorylation pathway.

## REFERENCES

1. Hall, C., et al. 1990. Novel human brain cDNA encoding a 34,000 M<sub>r</sub> protein n-chimaerin, related to both the regulatory domain of protein kinase C and BCR, the product of the breakpoint cluster region gene. *J. Mol. Biol.* 211: 11-16.
2. Drubin, D.G. 1991. Development of cell polarity in budding yeast. *Cell* 65: 1093-1096.
3. Hall, A. 1992. Ras-related GTPases and the cytoskeleton. *Mol. Biol. Cell* 3: 475-479.
4. Manser, E., et al. 1992. Diversity and versatility of GTPase activation proteins for the p21 Rho subfamily of Ras G proteins detected by a novel overlay assay. *J. Biol. Chem.* 267: 16025-16028.
5. Manser, E., et al. 1993. A non-receptor tyrosine kinase that inhibits the GTPase activity of p21<sup>Cdc42</sup>. *Nature* 363: 364-367.
6. Nur-E-Kamal, M.S., et al. 1999. The Cdc42-specific inhibitor derived from ACK-1 blocks v-Ha-Ras-induced transformation. *Oncogene* 18: 7787-7793.

## CHROMOSOMAL LOCATION

Genetic locus: Tnk2 (mouse) mapping to 16 B3.

## PRODUCT

ACK 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ACK shRNA Plasmid (m): sc-29633-SH and ACK shRNA (m) Lentiviral Particles: sc-29633-V as alternate gene silencing products.

For independent verification of ACK (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-29633A, sc-29633B and sc-29633C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

ACK siRNA (m) is recommended for the inhibition of ACK 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  $\mu$ M in 66  $\mu$ l. 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

ACK (A-11): sc-28336 is recommended as a control antibody for monitoring of ACK 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-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™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) 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® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ACK gene expression knockdown using RT-PCR Primer: ACK (m)-PR: sc-29633-PR (20  $\mu$ l). 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.