SANTA CRUZ BIOTECHNOLOGY, INC.

# RKIP siRNA (m): sc-36431

## BACKGROUND

Raf kinase inhibitor protein (RKIP) is a cytosolic protein that was initially characterized as a phosphatidylethanolamine-binding protein (PBP) expressed in brain tissue and secreted from testis fluid. In addition, RKIP was identified by yeast two-hybrid screening of human T cell libraries directed at identifying proteins that associate with the BXB kinase domain of the serine/threonine kinase, Raf-1. Subsequent *in vitro* and *in vivo* studies indicate that RKIP binds to both the active and inactive forms of Raf-1 and thereby regulates the signaling cascade of the MAP kinase pathway. The specific association of RKIP with kinase-active Raf-1 competitively inhibits the binding and activation of the Raf-1 substrate MEK. RKIP, in turn, affects downstream MAP kinase signaling by decreasing the activation of MEK effector proteins, including ERK1 and ERK2, and the subsequent induction of AP-1 mediated transcription.

## REFERENCES


1. Perry, A.C., et al. 1994. Sequence analysis of a mammalian phospholipid-binding protein from testis and epididymis and its distribution between spermatozoa and extracellular secretions. Biochem. J. 301: 235-242.
2. Minden, A., et al. 1994. Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. Science 266: 1719-1723.
3. Tohdoh, N., et al. 1995. Sequence homology of rat and human HCNP precursor proteins, bovine phosphatidylethanol-amine-binding protein and rat 23-kDa protein associated with the opioid-binding protein. Brain Res. Mol. Brain Res. 30: 381-384.
4. Kolch, W., et al. 1996. Inhibition of Raf-1 signaling by a monoclonal antibody, which interferes with Raf-1 activation and with Mek substrate binding. Oncogene 13:1305-1314.
5. Morrison, D.K. and Cutler, R.E. 1997. The complexity of Raf-1 regulation. Curr. Opin. Cell Biol. 9: 174-179.
6. Yeung, K., et al. 1999. Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. Nature 401: 173-177.


## CHROMOSOMAL LOCATION

Genetic locus: Pebp1 (mouse) mapping to 5 F.

## PRODUCT

RKIP 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 RKIP shRNA Plasmid (m): sc-36431-SH and RKIP shRNA (m) Lentiviral Particles: sc-36431-V as alternate gene silencing products.

For independent verification of RKIP (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-36431A, sc-36431B and sc-36431C.

## PROTOCOLS

See our web site at 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 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

RKIP siRNA (m) is recommended for the inhibition of RKIP 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

RKIP (D-5): sc-365973 is recommended as a control antibody for monitoring of RKIP 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 RKIP gene expression knockdown using RT-PCR Primer: RKIP (m)-PR: sc-36431-PR (20 µl, 503 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS


1. Han, S., et al. 2009. Integrated modulation of phorbol ester-induced Raf activation in EL4 lymphoma cells. Cell. Signal. 21: 793-800.


## RESEARCH USE

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