



RACK1 siRNA (r): sc-156032

BACKGROUND

Members of the protein kinase C (PKC) family play a key regulatory role in a variety of cellular functions, including cell growth and differentiation, gene expression, hormone secretion and membrane function. Receptor for activated C kinases, termed RACKs, are intracellular receptors for activated PKC that may be involved in the activation-induced translocation of PKC. RACK1 (receptor for activated C kinase 1) is a 317 amino acid G protein β subunit-like protein that functions as a RACK and inhibits the activity of Src tyrosine kinases. In response to PKC activation, the intracellular localization of RACK1 and PKC β changes, and RACK1 and PKC β co-localize to the same sites. RACK1 is therefore thought to be a shuttling protein for PKC β . A deficit in RACK1 may be associated with impaired PKC activation in the aging brain. The RACK1 gene is conserved in chimpanzee, canine, bovine, mouse, rat, chicken, zebrafish, fruit fly, mosquito, *C. elegans*, *S. pombe*, *S. cerevisiae*, *K. lactis*, *E. gossypii*, *M. grisea*, *N. crassa*, *A. thaliana*, rice and *P. falciparum*, and maps to human chromosome 5q35.3.

REFERENCES

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2. Castagna, M., et al. 1982. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J. Biol. Chem.* 257: 7847-7851.
3. Kikkawa, U., et al. 1983. Protein kinase C as a possible receptor of tumor-promoting phorbol esters. *J. Biol. Chem.* 258: 11442-11445.
4. Nishizuka, Y. 1984. The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* 308: 693-698.
5. Nishizuka, Y. 1984. Turnover of inositol phospholipids and signal transduction. *Science* 225: 1365-1370.
6. Ohno, S., et al. 1991. Structural and functional diversities of a family of signal transducing protein kinases, protein kinase C family; two distinct classes of PKC, conventional cPKC and novel nPKC. *Adv. Enzyme Regul.* 31: 287-303.
7. Olivier, A.R., et al. 1991. Expression and characterization of protein kinase C δ . *Eur. J. Biochem.* 200: 805-810.

CHROMOSOMAL LOCATION

Genetic locus: Gnb2l1 (rat) mapping to 10q21.

PRODUCT

RACK1 siRNA (r) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RACK1 shRNA Plasmid (r): sc-156032-SH and RACK1 shRNA (r) Lentiviral Particles: sc-156032-V as alternate gene silencing products.

For independent verification of RACK1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156032A, sc-156032B and sc-156032C.

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

APPLICATIONS

RACK1 siRNA (r) is recommended for the inhibition of RACK1 expression in rat 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 μ M in 66 μ 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

RACK1 (B-3): sc-17754 is recommended as a control antibody for monitoring of RACK1 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 RACK1 gene expression knockdown using RT-PCR Primer: RACK1 (r)-PR: sc-156032-PR (20 μ 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.

PROTOCOLS

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