

cGKII siRNA (r): sc-270328

BACKGROUND

cGKII (cGMP-dependent protein kinase type II) is a major receptor of intracellular cGMP and mediates a plethora of physiological responses. cGKII contains a conserved leucine zipper motif at the amino-terminus. It is expressed in small intestine, colon, prostate, and human brain tissues, and the cGKII gene maps to chromosome 4q21.21. cGKII has been shown to regulate the ion transport system in the intestine. Myristoylation of the penultimate glycine in cGKII appears to be essential for directing cGKII to the membrane, since cGKII is devoid of any hydrophobic transmembrane domains. The translocation of cGKII from the cytosol to the membrane allows it to function properly in regulating intestinal ion transport.

REFERENCES

1. Uhler, M.D. 1993. Cloning and expression of a novel cyclic GMP-dependent protein kinase from mouse brain. *J. Biol. Chem.* 268: 13586-13591.
2. Gamm, D.M., et al. 1995. The type II isoform of cGMP-dependent protein kinase is dimeric and possesses regulatory and catalytic properties distinct from the type I isoforms. *J. Biol. Chem.* 270: 27380-27388.
3. Vaandrager, A.B., et al. 1996. Signalling by cGMP-dependent protein kinases. *Mol. Cell. Biochem.* 157: 23-30.
4. Orstavik, S., et al. 1996. Molecular cloning, cDNA structure, and chromosomal localization of the human type II cGMP-dependent protein kinase. *Biochem. Biophys. Res. Commun.* 220: 759-765.
5. Vaandrager, A.B., et al. 1996. N-terminal myristoylation is required for membrane localization of cGMP-dependent protein kinase type II. *J. Biol. Chem.* 271: 7025-7029.
6. Wagner, C., et al. 1998. Role of cGMP-kinase II in the control of renin secretion and renin expression. *J. Clin. Invest.* 102: 1576-1582.
7. Francis, S.H., et al. 1999. Cyclic nucleotide-dependent protein kinases: intracellular receptors for cAMP and cGMP action. *Crit. Rev. Clin. Lab. Sci.* 36: 275-328.

CHROMOSOMAL LOCATION

Genetic locus: Prkg2 (rat) mapping to 14p22.

PRODUCT

cGKII 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 cGKII shRNA Plasmid (r): sc-270328-SH and cGKII shRNA (r) Lentiviral Particles: sc-270328-V as alternate gene silencing products.

For independent verification of cGKII (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-270328A, sc-270328B and sc-270328C.

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 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

cGKII siRNA (r) is recommended for the inhibition of cGKII 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

cGKII (D-3): sc-393126 is recommended as a control antibody for monitoring of cGKII 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 cGKII gene expression knockdown using RT-PCR Primer: cGKII (r)-PR: sc-270328-PR (20 μ l, 522 bp). 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.