

pki γ siRNA (h): sc-106417

BACKGROUND

The second messenger cyclic AMP (cAMP) mediates a diverse array of cellular responses such as proliferation, ion transport, regulation of metabolism and gene transcription by activating the cAMP-dependent protein kinase (cAPK or PKA). Protein kinase inhibitors are potent inhibitors of the catalytic subunit of PKAs. Pki γ (cAMP-dependent protein kinase inhibitor γ) is a 76 amino acid protein that inhibits catalytic subunit-dependent transcription and efficiently terminates nuclear PKA activity. Knockdown of the mRNA encoding pki γ substantially extends the anti-apoptotic effect of parathyroid hormone and β -adrenergic agonists. This evidence suggests that inhibition of pki γ activity may be a useful co-therapy in the treatment of osteoporosis. pki γ is highly expressed in skeletal muscle, testis and heart.

REFERENCES

- Collins, S.P. and Uhler, M.D. 1997. Characterization of pki γ , a novel isoform of the protein kinase inhibitor of cAMP-dependent protein kinase. *J. Biol. Chem.* 272: 18169-18178.
- Zheng, L., et al. 2000. Cloning and mapping of human PKIB and PKIG, and comparison of tissue expression patterns of three members of the protein kinase inhibitor family, including PKIA. *Biochem. J.* 349: 403-407.
- Taylor, S.S., et al. 2004. PKA: a portrait of protein kinase dynamics. *Biochim. Biophys. Acta* 1697: 259-269.
- Online Mendelian Inheritance in Man, OMIM[™]. 2004. Johns Hopkins University, Baltimore, MD. MIM Number: 604932. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
- Taylor, S.S., et al. 2005. Dynamics of signaling by PKA. *Biochim. Biophys. Acta* 1754: 25-37.
- Chen, X., et al. 2005. Endogenous protein kinase inhibitor γ terminates immediate-early gene expression induced by cAMP-dependent protein kinase (PKA) signaling: termination depends on PKA inactivation rather than PKA export from the nucleus. *J. Biol. Chem.* 280: 2700-2707.
- Dalton, G.D. and Dewey, W.L. 2006. Protein kinase inhibitor peptide (PKI): a family of endogenous neuropeptides that modulate neuronal cAMP-dependent protein kinase function. *Neuropeptides* 40: 23-34.

CHROMOSOMAL LOCATION

Genetic locus: PKIG (human) mapping to 20q13.12.

PRODUCT

pki γ siRNA (h) 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 pki γ shRNA Plasmid (h): sc-106417-SH and pki γ shRNA (h) Lentiviral Particles: sc-106417-V as alternate gene silencing products.

For independent verification of pki γ (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-106417A, sc-106417B and sc-106417C.

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

pki γ siRNA (h) is recommended for the inhibition of pki γ expression in human 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

pki γ (D-8): sc-515108 is recommended as a control antibody for monitoring of pki γ 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 pki γ gene expression knockdown using RT-PCR Primer: pki γ (h)-PR: sc-106417-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.