

PKA II β reg siRNA (m): sc-39167

BACKGROUND

The second messenger cyclic AMP (cAMP) mediates diverse cellular responses to external signals such as proliferation, ion transport, regulation of metabolism and gene transcription by activation of the cAMP-dependent protein kinase (cAPK or PKA). Activation of PKA occurs when cAMP binds to the two regulatory subunits of the tetrameric PKA holoenzyme resulting in release of active catalytic subunits. Three catalytic (C) subunits have been identified, designated C α , C β and C γ , that each represent specific gene products. C α and C β are closely related (93% amino acid sequence similarity), whereas C γ displays 83% and 79% similarity to C α and C β , respectively. Activation of transcription upon elevation of cAMP levels results from translocation of PKA to the nucleus where it phosphorylates the transcription factor cAMP response element binding protein (CREB) on serine 133 which in turn leads to TFIIIB binding to TATA-box-binding protein TBP1, thus linking phospho-CREB to the pol II transcription initiation complex.

REFERENCES

1. Wainwright, B., et al. 1987. A human regulatory subunit of type II cAMP-dependent protein kinase localized by its linkage relationship to several cloned chromosome 7q markers. *Cytogenet. Cell Genet.* 45: 237-239.
2. Levy, F.O., et al. 1988. Molecular cloning, complementary deoxyribonucleic acid structure and predicted full-length amino acid sequence of the hormone-inducible regulatory subunit of 3'-5'-cyclic adenosine monophosphate-dependent protein kinase from human testis. *Mol. Endocrinol.* 2: 1364-1373.
3. Solberg, R., et al. 1992. Mapping of the regulatory subunits RI β and RII β of cAMP-dependent protein kinase genes on human chromosome 7. *Genomics* 14: 63-69.
4. Adams, M.R., et al. 1997. Loss of haloperidol induced gene expression and catalepsy in protein kinase A-deficient mice. *Proc. Natl. Acad. Sci. USA* 94: 12157-12161.
5. Elliott, M.R., et al. 2004. Down-regulation of IL-2 production in T lymphocytes by phosphorylated protein kinase A-RII β . *J. Immunol.* 172: 7804-7812.

CHROMOSOMAL LOCATION

Genetic locus: Prkar2b (mouse) mapping to 12 A3.

PRODUCT

PKA II β reg 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PKA II β reg shRNA Plasmid (m): sc-39167-SH and PKA II β reg shRNA (m) Lentiviral Particles: sc-39167-V as alternate gene silencing products.

For independent verification of PKA II β reg (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-39167A, sc-39167B and sc-39167C.

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

PKA II β reg siRNA (m) is recommended for the inhibition of PKA II β reg 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 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

PKA II β reg (C-2): sc-376778 is recommended as a control antibody for monitoring of PKA II β reg 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 PKA II β reg gene expression knockdown using RT-PCR Primer: PKA II β reg (m)-PR: sc-39167-PR (20 μ l, 588 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.

PROTOCOLS

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