SANTA CRUZ BIOTECHNOLOGY, INC.

PKAβ cat siRNA (m): sc-39160

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 Cα, Cβ and Cγ, that each represent specific gene products. Cα and Cβ are closely related (93% amino acid sequence similarity), whereas Cγ displays 83% and 79% similarity to Cα and Cβ, 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 TFIIB binding to TATA-box-binding protein TBP1, thus linking p-CREB to the pol II transcription initiation complex.

REFERENCES

1. Beavo, J.A., et al. 1974. Activation of protein kinase by physiological concentrations of cyclic AMP. Proc. Natl. Acad. Sci. USA 71: 3580-3583.
2. Krebs, E.G., et al. 1980. Phosphorylation and dephosphorylation of enzymes. Annu. Rev. Biochem. 48: 923-959.
3. Maldonado, F., et al. 1988. cAMP-dependent protein kinase, α-catalytic subunit. Nucleic Acids Res. 16: 8189-8190.
4. Gonzalez, G.A., et al. 1989. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at Serine 133. Cell 59: 675-680.
5. Beebe, S.J., et al. 1990. cAMP-dependent protein kinase, β-catalytic subunit. Mol. Endocrinol. 4: 465-475.
6. Meinkoth, J.L., et al. 1993. Signal transduction through the cAMP-dependent protein kinase. Mol. Cell. Biochem. 127/128: 179-186.
7. Nordheim, A. 1994. CREB takes CBP to tango. Nature 370: 177-178.

CHROMOSOMAL LOCATION

Genetic locus: Prkacb (mouse) mapping to 3 H2.

PRODUCT

PKAβ cat 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 PKAβ cat shRNA Plasmid (m): sc-39160-SH and PKAβ cat shRNA (m) Lentiviral Particles: sc-39160-V as alternate gene silencing products.

For independent verification of PKAβ cat (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-39160A, sc-39160B and sc-39160C.

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

PKAβ cat siRNA (m) is recommended for the inhibition of PKAβ cat 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

PKAα/β/γ cat (B-4): sc-365615 is recommended as a control antibody for monitoring of PKAβ cat 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 PKAβ cat gene expression knockdown using RT-PCR Primer: PKAβ cat (m)-PR: sc-39160-PR (20 µl, 568 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.