SANTA CRUZ BIOTECHNOLOGY, INC.

PKAα cat siRNA (h): sc-36240

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 A (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 PKAα cat (Cα), PKAβ cat (Cβ) and PKAγ cat (Cγ). Each subunit represents specific gene products. PKAα cat and PKAβ cat are closely related (93% amino acid sequence similarity), whereas PKAγ cat displays 83% and 79% similarity to PKAα cat and PKAβ cat, 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 phospho-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. 1979. Phosphorylation and dephosphorylation of enzymes. Annu. Rev. Biochem. 48: 923-959.

CHROMOSOMAL LOCATION

Genetic locus: PRKACA (human) mapping to 19p13.12.

PRODUCT

PKAα cat 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PKAα cat shRNA Plasmid (h): sc-36240-SH and PKAα cat shRNA (h) Lentiviral Particles: sc-36240-V as alternate gene silencing products.

For independent verification of PKAα cat (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-36240A, sc-36240B and sc-36240C.

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

PKAα cat siRNA (h) is recommended for the inhibition of PKAα cat 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 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 cat (A-2): sc-28315 is recommended as a control antibody for monitoring of PKAα 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PKAα cat gene expression knockdown using RT-PCR Primer: PKAα cat (h)-PR: sc-36240-PR (20 µl, 497 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Hu, A., et al. 2008. Prolonged heterologous β_2-adrenoceptor desensitization promotes proasthmatic airway smooth muscle function via PKA/ERK1/2-mediated phosphodiesterase-4 induction. Am. J. Physiol. Lung Cell. Mol. Physiol. 294: L1055-L1067.
2. Kaya, A.I., et al. 2012. Cell contact-dependent functional selectivity of β_2-adrenergic receptor ligands in stimulating cAMP accumulation and extracellular signal-regulated kinase phosphorylation. J. Biol. Chem. 287: 6362-6374.
3. Hedrick, E.D., et al. 2013. Differential PKA activation and AKAP association determines cell fate in cancer cells. J. Mol. Signal. 8: 10.
4. Hwang, S., et al. 2015. CCN1 acutely increases nitric oxide production via Integrin $\alpha_v\beta_3$-Akt-S6K-phosphorylation of endothelial nitric oxide synthase at the serine 1177 signaling axis. Free Radic. Biol. Med. 89: 229-240.
5. Pedram, A., et al. 2016. Membrane and nuclear estrogen receptor α collaborate to suppress adipogenesis but not triglyceride content. FASEB J. 30: 230-240.
6. Hedrick, E., et al. 2018. TGFβ-induced lung cancer cell migration is NR4A1-dependent. Mol. Cancer Res. 16: 1991-2002.
7. Jia, Y., et al. 2023. Tripartite motif containing 69 elicits ERK2-dependent EYA4 turnover to impart pancreatic tumorigenesis. J. Cancer 14: 200-218.

RESEARCH USE

For research use only, not for use in diagnostic procedures.