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SANTA CRUZ BIOTECHNOLOGY, INC.

Clock siRNA (r): sc-270115

BACKGROUND

Biological timepieces called circadian clocks are responsible for the regulation of hormonal rhythms, sleep cycles and other behaviors. The superchiasmatic nucleus (SCN), which is located in the brain, was the first mammalian circadian clock to be discovered. Clock, a member of the basic-helix-loop-helix-PAS (bHLH-PAS) family of transcription factors, has also been identified as having circadian. Mutations within the Clock gene have been shown to increase the length of the endogenous period and to cause a loss of rhythmicity of circadian oscillations. Clock contains a DNA-binding domain, a protein dimerization domain and a glutamine-rich C-terminal region, which indicates transactivation capabilities. It has been speculated that Clock may regulate circadian rhythmicity in combination with other proteins such as Per. Per is also a PAS-domain containing protein that exhibits circadian function. Highest expression of Clock is seen in the hypothalamus and the eye.

REFERENCES

1. Morell, V. 1995. A 24-hour circadian Clock is found in the mammalian retina. Science 272: 349.
2. King, D.P., et al. 1997. The mouse Clock mutation behaves as an antimorph and maps within the W19H deletion, distal of Kit. Genetics 146: 1049-1060.
3. Reppert, S.M., et al. 1997. Forward genetic approach strikes gold: cloning of a mammalian Clock gene. Cell 89: 487-490.
4. King, D.P., et al. 1997. Positional cloning of the mouse circadian Clock gene. Cell 89: 641-653.
5. Antoch, M.P., et al. 1997. Functional identification of the mouse circadian Clock gene by transgenic BAC rescue. Cell 89: 655-667.
6. Tei, H., et al. 1997. Circadian oscillation of a mammalian homologue of the *Drosophila* period gene. Nature 389: 512-516.
7. Kondratov, R.V., et al. 2003. BMAL1-dependent circadian oscillation of nuclear Clock: posttranslational events induced by dimerization of transcriptional activators of the mammalian Clock system. Genes Dev. 17: 1921-1932.
8. Malatesta, M., et al. 2003. Fine distribution of Clock protein in hepatocytes of hibernating dormice. Eur. J. Histochem. 47: 233-240.

CHROMOSOMAL LOCATION

Genetic locus: Clock (rat) mapping to 14p11.

PRODUCT

Clock 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Clock shRNA Plasmid (r): sc-270115-SH and Clock shRNA (r) Lentiviral Particles: sc-270115-V as alternate gene silencing products.

For independent verification of Clock (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-270115A, sc-270115B and sc-270115C.

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

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

Clock (C-8): sc-271603 is recommended as a control antibody for monitoring of Clock 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 Clock gene expression knockdown using RT-PCR Primer: Clock (r)-PR: sc-270115-PR (20 µl, 565 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Tsukamoto-Yamauchi, N., et al. 2015. Interaction of pituitary hormones and expression of Clock genes modulated by bone morphogenetic protein-4 and melatonin. Biochem. Biophys. Res. Commun. 459: 172-177.

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.