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SANTA CRUZ BIOTECHNOLOGY, INC.

CRY1 siRNA (h): sc-43706

BACKGROUND

Circadian clocks are biological timepieces that regulate hormonal rhythms, sleep cycles and feeding behaviors. These rhythms are generated in the superchiasmatic nucleus (SCN), a cell-autonomous circadian oscillator located within the brain that is synchronized with the environment by light. A number of transcription factors, including Clock and BMAL1, are molecular components of the SCN that induce the expression of proteins involved in light/dark cycle entrainment, which include Per1 and Per2. Tim, for timeless, generates a negative feedback loop that regulates the activity of Clock by suppressing the expression of Clock target genes. Tim forms heterodimers with Per1 and Per2 that bind Clock and block the activation of CLOCK-BMAL1 dimers to to repress Per gene expression. Additionally, the CRY proteins, which are cryptochrome photoreceptors for the circadian clock, function as light-independent inhibitors of the circadian clock. CRY1 and CRY2 negatively regulate SCN components by associating with the activators, Clock-BMAL1, and also with the various feedback inhibitors Per1, Per2 and Tim.

REFERENCES

1. Morell, V. 1996. A 24-hour circadian clock is found in the mammalian retina. Science 272: 349.
2. Albrecht, U., et al. 1997. A differential response of two putative mammalian circadian regulators, mPer1 and mPer2, to light. Cell 91: 1055-1064.
3. Sangoram, A.M., et al. 1998. Mammalian circadian autoregulatory loop: a timeless ortholog and mPer1 interact and negatively regulate CLOCK-BMAL1-induced transcription. Neuron 21: 1101-1113.
4. Zylka, M.J., et al. 1998. Molecular analysis of mammalian timeless. Neuron 21: 1115-1122.
5. Jin, X., et al. 1999. A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. Cell 96: 57-68.
6. Dunlap, J.C. 1999. Molecular bases for circadian clocks. Cell 96: 271-290.
7. Griffin, E.A., et al. 1999. Light-independent role of CRY1 and CRY2 in the mammalian circadian clock. Science 286: 768-771.

CHROMOSOMAL LOCATION

Genetic locus: CRY1 (human) mapping to 12q23.3.

PRODUCT

CRY1 siRNA (h) is a pool of 2 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 CRY1 shRNA Plasmid (h): sc-43706-SH and CRY1 shRNA (h) Lentiviral Particles: sc-43706-V as alternate gene silencing products.

For independent verification of CRY1 (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-43706A and sc-43706B.

RESEARCH USE

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

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

CRY1 siRNA (h) is recommended for the inhibition of CRY1 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

CRY1 (H-12): sc-393466 is recommended as a control antibody for monitoring of CRY1 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 CRY1 gene expression knockdown using RT-PCR Primer: CRY1 (h)-PR: sc-43706-PR (20 µl, 548 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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