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CRY2 shRNA (m) Lentiviral Particles: sc-44836-V

BACKGROUND

Circadian clocks are biological timepieces that regulate hormonal rhythms, sleep cycles and feeding behaviors. These rhythms are generated in the suprachiasmatic 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 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

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- 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.
- Zylka, M.J., et al. 1998. Molecular analysis of mammalian timeless. *Neuron* 21: 1115-1122.
- Jin, X., et al. 1999. A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. *Cell* 96: 57-68.
- Dunlap, J.C. 1999. Molecular bases for circadian clocks. *Cell* 96: 271-290.
- 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: Cry2 (mouse) mapping to 2 E1.

PRODUCT

CRY2 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CRY2 siRNA (m): sc-44836 and CRY2 shRNA Plasmid (m): sc-44836-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

CRY2 shRNA (m) Lentiviral Particles is recommended for the inhibition of CRY2 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

CRY2 (A-20): sc-5954 is recommended as a control antibody for monitoring of CRY2 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker[™] compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CRY2 gene expression knockdown using RT-PCR Primer: CRY2 (m)-PR: sc-44836-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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