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CRY2 siRNA (m): sc-44836

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

- Morell, V. 1996. A 24-hour circadian clock is found in the mammalian retina. *Science* 272: 349.
- Albrecht, U., et al. 1997. A differential response of two putative mammalian circadian regulators, mPer1 and mPer2, to light. *Cell* 91: 1055-1064.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: Cry2 (mouse) mapping to 2 E1.

PRODUCT

CRY2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CRY2 shRNA Plasmid (m): sc-44836-SH and CRY2 shRNA (m) Lentiviral Particles: sc-44836-V as alternate gene silencing products.

For independent verification of CRY2 (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-44836A, sc-44836B and sc-44836C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CRY2 siRNA (m) is recommended for the inhibition of CRY2 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 μ M in 66 μ l. 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

CRY2 (181J2J): sc-517631 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 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.

SELECT PRODUCT CITATIONS

- Gaddameedhi, S., et al. 2012. Effect of circadian clock mutations on DNA damage response in mammalian cells. *Cell Cycle* 11: 3481-3491.

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.