

Timeless siRNA (m): sc-72017

BACKGROUND

Biological timepieces called circadian clocks are responsible for the regulation of hormonal rhythms, sleep cycles and other behaviors. The suprachiasmatic nucleus (SCN), which is located in the brain, was the first mammalian circadian clock to be discovered. A number of transcription factors appearing to be molecular components of the SCN clock have been identified. Mutations within the Clock gene increase the length of the endogenous period and cause a loss of rhythmicity of circadian oscillations. Three mammalian period proteins, designated Per1, Per2 and Per3, exhibit circadian rhythms in the SCN. During subjective night, Per1 and Per2 RNA levels increase in response to light pulses, while Per3 RNA levels show no change in response to light pulses. Timeless interacts with Per1 as well as Per2. Timeless and Per1 negatively regulate Clock-BMAL1-induced transcription.

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. Antoch, M.P., et al. 1997. Functional identification of the mouse circadian Clock gene by transgenic BAC rescue. *Cell* 89: 655-667.
4. Zylka, M.J., et al. 1998. Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain. *Neuron* 20: 1103-1110.
5. 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.

CHROMOSOMAL LOCATION

Genetic locus: Timeless (mouse) mapping to 10 D3.

PRODUCT

Timeless 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 Timeless shRNA Plasmid (m): sc-72017-SH and Timeless shRNA (m) Lentiviral Particles: sc-72017-V as alternate gene silencing products.

For independent verification of Timeless (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-72017A, sc-72017B and sc-72017C.

RESEARCH USE

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

PROTOCOLS

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

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

Timeless siRNA (m) is recommended for the inhibition of Timeless 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

Timeless (A-3): sc-393122 is recommended as a control antibody for monitoring of Timeless 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Timeless gene expression knockdown using RT-PCR Primer: Timeless (m)-PR: sc-72017-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.