

# Timeless siRNA (r): sc-270603

## 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. Tim, for timeless, interacts with Per1 as well as Per2; and Tim and Per1 negatively regulate Clock-BMAL1-induced transcription.

## REFERENCES

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## CHROMOSOMAL LOCATION

Genetic locus: Timeless (rat) mapping to 7q11.

## PRODUCT

Timeless 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Timeless shRNA Plasmid (r): sc-270603-SH and Timeless shRNA (r) Lentiviral Particles: sc-270603-V as alternate gene silencing products.

For independent verification of Timeless (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-270603A, sc-270603B and sc-270603C.

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

## APPLICATIONS

Timeless siRNA (r) is recommended for the inhibition of Timeless 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  $\mu$ M in 66  $\mu$ 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 reagents are recommended: 1) Western Blotting: use m-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 Timeless gene expression knockdown using RT-PCR Primer: Timeless (r)-PR: sc-270603-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.