

# ORC4 siRNA (h): sc-38157

## BACKGROUND

The initiation of DNA replication is a multi-step process that depends on the formation of pre-replication complexes, which trigger initiation. Among the proteins required for establishing these complexes are the origin recognition complex (ORC) proteins. ORC proteins bind specifically to origins of replication where they serve as scaffold for the assembly of additional initiation factors. Human ORC subunits 1-6 are expressed in the nucleus of proliferating cells and tissues, such as the testis. ORC1 and ORC2 are both expressed at equivalent concentrations throughout the cell cycle; however, only ORC2 remains stably bound to chromatin. ORC4 and ORC6 are also expressed constantly throughout the cell cycle. ORC2, ORC3, ORC4 and ORC5 form a core complex upon which the ORC6 and ORC1 assemble. The formation of this core complex suggests ORC proteins play a crucial role in the G<sub>1</sub>-S transition in mammalian cells.

## REFERENCES

1. Quintana, D.G., et al 1997. Identification of the HsORC4, a member of the human origin of replication recognition complex. *J. Biol. Chem.* 272: 28247-28251.
2. Mendez, J. and Stillman, B. 2000. Chromatin association of human origin recognition complex, Cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. *Mol. Cell. Biol.* 20: 8602-8612.
3. Dhar, S.K. et al. 2000. Identification and characterization of the human ORC6 homolog. *J. Biol. Chem.* 275: 34983-34988.
4. Thome, K.C., et al. 2000. Subsets of human origin recognition complex (ORC) subunits are expressed in non-proliferating cells and associate with non-ORC proteins. *J. Biol. Chem.* 275: 35233-35241.
5. Kreitz, S., et al. 2000. The human origin-recognition-complex protein 1 dissociates from chromatin during S phase in HeLa cells. *J. Biol. Chem.* 276: 6337-6342.
6. Natale, D.A., et al. 2000. Selective instability of ORC1 protein accounts for the absence of functional origin recognition complexes during the M-G<sub>1</sub> transition in mammals. *EMBO J.* 19: 2728-2738.

## CHROMOSOMAL LOCATION

Genetic locus: ORC4 (human) mapping to 2q22.3.

## PRODUCT

ORC4 siRNA (h) 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 ORC4 shRNA Plasmid (h): sc-38157-SH and ORC4 shRNA (h) Lentiviral Particles: sc-38157-V as alternate gene silencing products.

For independent verification of ORC4 (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-38157A, sc-38157B and sc-38157C.

## 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

ORC4 siRNA (h) is recommended for the inhibition of ORC4 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  $\mu$ 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

ORC4 (H-2): sc-398455 is recommended as a control antibody for monitoring of ORC4 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 ORC4 gene expression knockdown using RT-PCR Primer: ORC4 (h)-PR: sc-38157-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.