

# MtRPOL siRNA (h): sc-61099

## BACKGROUND

The circular mitochondrial genome contains 37 genes that encode the RNA constituents of the mitochondrial translational apparatus. Gene expression in mitochondria relies upon several nuclear genes that encode protein components required for transcription and translation of MtDNA-encoded genes, as well as protein and RNA components necessary for MtDNA replication. Mitochondrial RNA polymerase (MtRPOL) modulates gene expression in the mitochondria by providing the RNA primers for replication/initiation. It also participates in the maintenance and propagation of the mitochondrial genome. Genes involved in the replication and expression of MtRPOL may be candidates for various human disorders. MtRPOL consists of 1,230 amino acid residues, the sequence of which demonstrates substantial homology with sequences corresponding to mitochondrial RNA polymerases from lower eukaryotes and to RNA polymerases from several bacteriophages.

## REFERENCES

1. Anderson, S., et al. 1981. Sequence and organization of the human mitochondrial genome. *Nature* 290: 457-465.
2. Humphery-Smith, I., et al. 1995. Polypeptide cartography of *Spiroplasma taiwanense*. *Electrophoresis* 15: 1212-1217.
3. Wasinger, V.C., et al. 1996. Progress with gene-product mapping of the Mollicutes: *Mycoplasma genitalium*. *Electrophoresis* 16: 1090-1494.
4. Tiranti, V., et al. 1997. Identification of the gene encoding the human mitochondrial RN (h-mtRPOL) by cyberscreening of the Expressed Sequence Tags database. *Hum. Mol. Genet.* 6: 615-625.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 601778. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
6. Fish, J., et al. 2004. Discovery of a major D-loop replicati synthesis. *Science* 306: 2098-2101.
7. Kravchenko, J.E., et al. 2005. Transcription of mammalian messenger RNAs by a nuclear RNA polymerase of mitochondrial origin. *Nature* 436: 735-739.

## CHROMOSOMAL LOCATION

Genetic locus: POLRMT (human) mapping to 19p13.3.

## PRODUCT

MtRPOL 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 MtRPOL shRNA Plasmid (h): sc-61099-SH and MtRPOL shRNA (h) Lentiviral Particles: sc-61099-V as alternate gene silencing products.

For independent verification of MtRPOL (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-61099A, sc-61099B and sc-61099C.

## 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

MtRPOL siRNA (h) is recommended for the inhibition of MtRPOL 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

MtRPOL (B-1): sc-365082 is recommended as a control antibody for monitoring of MtRPOL 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 MtRPOL gene expression knockdown using RT-PCR Primer: MtRPOL (h)-PR: sc-61099-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.