SANTA CRUZ BIOTECHNOLOGY, INC.

RNGTT siRNA (h): sc-95119

BACKGROUND

RNGTT (RNA guanylyltransferase and 5'-phosphatase), also known as HCE, HCE1, hCAP or CAP1A, is a 597 amino acid protein that localizes to the nucleus and exists as four alternatively spliced isoforms. Expressed in kidney, lung, heart, brain, liver, testis, skin and muscle, RNGTT acts as a bifunctional mRNA-capping enzyme that exhibits RNA 5'-triphosphatase activity at its N-terminus and mRNA guanylyltransferase activity at its C-terminus. Via its catalytic activity, RNGTT facilitates the first two steps of mRNA cap formation, naming the removal of a γ-phosphate from the end of nascent mRNA and the subsequent transfer of the phospho residue to the 5'-diphosphate terminus. The gene encoding RNGTT maps to human chromosome 6, which contains 170 million base pairs and comprises nearly 6% of the human genome.

REFERENCES

1. Yue, Z., et al. 1997. Mammalian capping enzyme complements mutant *Saccharomyces cerevisiae* lacking mRNA guanylyltransferase and selectively binds the elongating form of RNA polymerase II. Proc. Natl. Acad. Sci. USA 94: 12898-12903.
2. Tsukamoto, T., et al. 1998. Cloning and characterization of two human cDNAs encoding the mRNA capping enzyme. Biochem. Biophys. Res. Commun. 243: 101-108.
3. Tsukamoto, T., et al. 1998. Cloning and characterization of three human cDNAs encoding mRNA (guanine-7-)-methyltransferase, an mRNA cap methylase. Biochem. Biophys. Res. Commun. 251: 27-34.
4. Pillutla, R.C., et al. 1998. Human mRNA capping enzyme (RNGTT) and cap methyltransferase (RNMT) map to 6q16 and 18p11.22-p11.23, respectively. Genomics 54: 351-353.
5. Pillutla, R.C., et al. 1998. Recombinant human mRNA cap methyltransferase binds capping enzyme/RNA polymerase IIo complexes. J. Biol. Chem. 273: 21443-21446.
6. Yamada-Okabe, T., et al. 1998. Isolation and characterization of a human cDNA for mRNA 5'-capping enzyme. Nucleic Acids Res. 26: 1700-1706.
7. Online Mendelian Inheritance in Man, OMIM™. 1999. Johns Hopkins University, Baltimore, MD. MIM Number: 603512. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: RNGTT (human) mapping to 6q15.

PRODUCT

RNGTT 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RNGTT shRNA Plasmid (h): sc-95119-SH and RNGTT shRNA (h) Lentiviral Particles: sc-95119-V as alternate gene silencing products.

For independent verification of RNGTT (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-95119A, sc-95119B and sc-95119C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

RNGTT siRNA (h) is recommended for the inhibition of RNGTT 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 µM in 66 µl. 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

RNGTT (D-3): sc-377464 is recommended as a control antibody for monitoring of RNGTT 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 RNGTT gene expression knockdown using RT-PCR Primer: RNGTT (h)-PR: sc-95119-PR (20 µl). 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 for detailed protocols and support products.