SANTA CRUZ BIOTECHNOLOGY, INC.

RNase H1 siRNA (m): sc-152994

BACKGROUND

The human RNase H1 enzyme is a cytoplasmic endonuclease that degrades the RNA of RNA-DNA hybrids resulting in 5'-phosphomonoester products. Mn^{2+} and N-ethylmaleimide can inhibit Mg^{2+}-dependent RNase H1 activity. The RNase H1 gene is present at similar levels in all human cells and tissues, indicating that RNase H1 may be a housekeeping protein. The human RNase H1 gene maps to chromosome 2p25.3 with pseudogenes present on chromosome 17p11.2 and chromosome 1q.

REFERENCES

1. Wu, H., et al. 1998. Molecular cloning and expression of cDNA for human RNase H. Antisense Nucleic Acid Drug Dev. 8: 53-61.
2. Cerritelli, S., et al. 1998. Cloning, expression, and mapping of ribonucleases H of human and mouse related to bacterial RNase H1. Genomics 53: 300-307.
3. ten Asbroek, A., et al. 2002. Ribonuclease H1 maps to chromosome 2 and has at least three pseudogene loci in the human genome. Genomics 79: 818-823.
4. Lima, W.F., et al. 2003. Human RNase H1 activity is regulated by a unique redox switch formed between adjacent cysteines. J. Biol. Chem. 278: 14906-14912.
5. Lima, W.F., et al. 2003. Human RNase H1 uses one tryptophan and two lysines to position the enzyme at the 3'-DNA/5'-RNA terminus of the heteroduplex substrate. J. Biol. Chem. 278: 49860-49867.
6. Wu, H., et al. 2004. Determination of the role of the human RNase H1 in the pharmacology of DNA-like antisense drugs. J. Biol. Chem. 279: 17181-17189.
7. Lima, W.F., et al. 2004. Structural requirements at the catalytic site of the heteroduplex substrate for human RNase H1 catalysis. J. Biol. Chem. 279: 36317-36326.

CHROMOSOMAL LOCATION

Genetic locus: Rnaseh1 (mouse) mapping to 12 A2.

PRODUCT

RNase H1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RNase H1 shRNA Plasmid (m): sc-152994-SH and RNase H1 shRNA (m) Lentiviral Particles: sc-152994-V as alternate gene silencing products.

For independent verification of RNase H1 (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-152994A, sc-152994B and sc-152994C.

PROTOCOLS

See our web site at www.scbt.com 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 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

RNase H1 siRNA (m) is recommended for the inhibition of RNase H1 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 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

RNase H1 (H-4): sc-376326 is recommended as a control antibody for monitoring of RNase H1 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 RNase H1 gene expression knockdown using RT-PCR Primer: RNase H1 (m)-PR: sc-152994-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.