

RNase HII-A siRNA (h): sc-62954

BACKGROUND

The RNase HII complex is an endonuclease that degrades RNA found in RNA:DNA duplexes and is composed of one catalytic subunit and two non-catalytic subunits. RNase HII-A, also called RNASEH2A (ribonuclease H2 subunit A), RNASEHI, AGS4 or RNHIA, is the 299 amino acid catalytic subunit of RNase HII. Localized to the nucleus, RNase HII-A mediates the removal of Okazaki fragment RNA primers that are present on the lagging strand during DNA replication. RNase HII-A catalyzes the endonucleolytic cleavage of RNA to a 5'-phosphomonoester and is able to bind magnesium or manganese as cofactors. Defects in the gene encoding RNase HII-A are the cause of Aicardi-Goutieres syndrome type 4 (AGS4), an autosomal recessive encephalopathy characterized by cerebral atrophy, leukodystrophy, intracranial calcifications and chronic cerebrospinal fluid (CSF) lymphocytosis. Patients affected by AGS4 have severe neurological dysfunctions and often die in early childhood.

REFERENCES

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2. ten Asbroek, A.L., et al. 2002. The involvement of human ribonucleases H1 and H2 in the variation of response of cells to antisense phosphorothioate oligonucleotides. *Eur. J. Biochem.* 269: 583-592.
3. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606034. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Jeong, H.S., et al. 2004. RNase H2 of *Saccharomyces cerevisiae* is a complex of three proteins. *Nucleic Acids Res.* 32: 407-414.
5. Bayliss, C.D., et al. 2005. Destabilization of tetranucleotide repeats in *Haemophilus influenzae* mutants lacking RNase HI or the Klenow domain of PolI. *Nucleic Acids Res.* 33: 400-408.
6. Crow, Y.J., et al. 2006. Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutières syndrome and mimic congenital viral brain infection. *Nat. Genet.* 38: 910-916.

CHROMOSOMAL LOCATION

Genetic locus: RNASEH2A (human) mapping to 19p13.2.

PRODUCT

RNase HII-A 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RNase HII-A shRNA Plasmid (h): sc-62954-SH and RNase HII-A shRNA (h) Lentiviral Particles: sc-62954-V as alternate gene silencing products.

For independent verification of RNase HII-A (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-62954A, sc-62954B and sc-62954C.

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

APPLICATIONS

RNase HII-A siRNA (h) is recommended for the inhibition of RNase HII-A 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 μ M in 66 μ 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

RNase HII-A (G-10): sc-515475 is recommended as a control antibody for monitoring of RNase HII-A 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 HII-A gene expression knockdown using RT-PCR Primer: RNase HII-A (h)-PR: sc-62954-PR (20 μ 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 for detailed protocols and support products.