SANTA CRUZ BIOTECHNOLOGY, INC.

ADAT1 siRNA (m): sc-37662

BACKGROUND

Editing of RNA alters the nucleotide sequence of a transcript to produce codon changes, which can result in alternative translation patterns from a single pre-mRNA. One type of RNA editing involves tRNA-specific adenosine deaminase, ADAT1, which is responsible for the first step in the processing of eukaryotic tRNAAla transcripts that undergo specific adenosine to inosine modifications. Additionally, members of the double-stranded RNA (dsRNA) adenosine deaminase family of enzymes, ADAR1 and ADAR2, act on double-stranded regions of RNA. dsRNA structures are formed by base pairing of an exonic sequence around the editing site with a complementary sequence in the downstream intron. ADAR family member-mediated editing occurs in the nucleus before splicing removes the respective intron. These enzymes all faciliate the deamination of adenosine to generate inosine, which is then translated as guanosine. ADAR1, ADAR2 and a related brain-specific ADAR family member, RED2, contain a central series of double-stranded RNA-binding motifs and a C-terminal catalytic domain. ADAR1 also contains a novel Zα-DNA binding domain at the N-terminal region, and when bound to Z-DNA-ADAR1 is substantially less susceptible to proteolytic degradation.

REFERENCES

1. Maas, S., et al. 1996. Structural requirements for RNA editing in glutamate receptor pre-mRNAs by recombinant double-stranded RNA adenosine deaminase. J. Biol. Chem. 271: 12221-12226.
2. Melcher, T., et al. 1996. RED2, a brain-specific member of the RNA-specific adenosine deaminase family. J. Biol. Chem. 271: 31795-31798.
3. Rueter, S.M., et al. 1999. Regulation of alternative splicing by RNA editing. Nature 399: 75-80.
4. Maas, S., et al. 1999. Identification and characterization of a human tRNA-specific adenosine deaminase related to the ADAR family of pre-mRNA editing enzymes. Proc. Natl. Acad. Sci. USA 96: 8895-8900.
5. Lehmann, K.A., et al. 1999. The importance of internal loops within RNA substrates of ADAR1. J. Mol. Biol. 291: 1-13.
6. Schade, M., et al. 1999. The solution structure of the Zα domain of the human RNA editing enzyme ADAR1 reveals a prepositioned binding surface for Z-DNA. Proc. Natl. Acad. Sci. USA 96: 12465-12470.

CHROMOSOMAL LOCATION

Genetic locus: Adat1 (mouse) mapping to 8 E1.

PRODUCT

ADAT1 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 ADAT1 shRNA Plasmid (m): sc-37662-SH and ADAT1 shRNA (m) Lentiviral Particles: sc-37662-V as alternate gene silencing products.

For independent verification of ADAT1 (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-37662A, sc-37662B and sc-37662C.

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

ADAT1 siRNA (m) is recommended for the inhibition of ADAT1 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

ADAT1 (C-5): sc-271812 is recommended as a control antibody for monitoring of ADAT1 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 ADAT1 gene expression knockdown using RT-PCR Primer: ADAT1 (m)-PR: sc-37662-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.