

ADAR1 siRNA (h): sc-37657

BACKGROUND

RNA-specific adenosine deaminase (ADAR1, DSH, IFI4, p136, DRADA, DSRAD, K88dsRBP) mediates RNA editing by destabilizing double stranded RNA through deamination of adenosine to inosine in structured or double-stranded RNAs. ADAR1 is expressed from an interferon-response promoter and has a Z-DNA/Z-RNA binding domain at its N-terminus. ADAR1 co-localizes with SUMO-1 in a subnucleolar region that is distinct from the fibrillar center, the dense fibrillar component and the granular component. Localization of nuclear ADAR1 is under the influence of a nucleolar localization signal (NoLS) in the middle of ADAR1 and the exporting activity of the nuclear exporter signal (NES) near the N terminus. ADAR1 upregulates nuclear factor 90 (NF90)-mediated gene expression by interacting with NF110, NF90 and NF45. ADAR1 binds short interfering RNA (siRNA), and gene silencing by siRNA is significantly more effective in mouse fibroblasts homozygous for an ADAR1 null mutation than in wild-type cells. ADAR1 may limit the efficacy of siRNA in mammalian cells.

REFERENCES

1. Strehblow, A., et al. 2002. Nucleocytoplasmic distribution of human RNA-editing enzyme ADAR1 is modulated by double-stranded RNA-binding domains, a leucine-rich export signal, and a putative dimerization domain. *Mol. Biol. Cell* 13: 3822-3835.
2. Herbert, A., et al. 2002. Induction of protein translation by ADAR1 within living cell nuclei is not dependent on RNA editing. *Mol. Cell* 10: 1235-1246.
3. Nie, Y., et al. 2004. Subcellular distribution of ADAR1 isoforms is synergistically determined by three nuclear discrimination signals and a regulatory motif. *J. Biol. Chem.* 279: 13249-13255.

CHROMOSOMAL LOCATION

Genetic locus: ADAR (human) mapping to 1q21.3.

PRODUCT

ADAR1 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 ADAR1 shRNA Plasmid (h): sc-37657-SH and ADAR1 shRNA (h) Lentiviral Particles: sc-37657-V as alternate gene silencing products.

For independent verification of ADAR1 (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-37657A, sc-37657B and sc-37657C.

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

ADAR1 siRNA (h) is recommended for the inhibition of ADAR1 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

ADAR1 (D-8): sc-271854 is recommended as a control antibody for monitoring of ADAR1 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 λ BP-HRP: sc-516132 or m-IgG λ BP-HRP (Cruz Marker): sc-516132-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 λ BP-FITC: sc-516185 or m-IgG λ BP-PE: sc-516186 (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 ADAR1 gene expression knockdown using RT-PCR Primer: ADAR1 (h)-PR: sc-37657-PR (20 μ l, 553 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Daou, S., et al. 2020. A phenolic small molecule inhibitor of RNase L prevents cell death from ADAR1 deficiency. *Proc. Natl. Acad. Sci. USA* 117: 24802-24812.
2. Sheikh, T.N., et al. 2021. Growth inhibition and induction of innate immune signaling of chondrosarcomas with epigenetic inhibitors. *Mol. Cancer Ther.* 20: 2362-2371.

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.