SANTA CRUZ BIOTECHNOLOGY, INC.

ARD siRNA (h): sc-72527

BACKGROUND

ARD (acireductone dioxygenase), also known as ADI1, APL1, SIPL, SIP-L or MTCBP1, is a 179 amino acid protein that localizes to the nucleus, as well as to the cytoplasmic side of the cell membrane, and belongs to the acireductone dioxygenase family of metal-binding enzymes. Expressed in brain, heart, lung, colon, liver, kidney, spleen and skeletal muscle, ARD uses nickel as a cofactor to catalyze a crucial step in the L-methionine biosynthetic pathway, namely the creation of L-methionine from (S)-methyl-5-thio-α-D-ribose 1-phosphate. Additionally, ARD interacts with MT-MMP-1 and may be able to down-regulate MT-MMP-1-mediated cell migration. Multiple isoforms of ARD exist due to alternative splicing events.

REFERENCES

1. Yeh, C.T., et al. 2001. Identification of a hepatic factor capable of supporting hepatitis C virus replication in a nonpermissive cell line. J. Virol. 75: 11017-11024.
2. Uekita, T., et al. 2004. Membrane-type 1 matrix metalloproteinase cytoplasmic tail-binding protein-1 is a new member of the Cupin superfamily. A possible multifunctional protein acting as an invasion suppressor down-regulated in tumors. J. Biol. Chem. 279: 12734-12743.
3. Yamada, S., et al. 2004. Expression profiling and differential screening between hepatoblastomas and the corresponding normal livers: identification of high expression of the PLK1 oncogene as a poor-prognostic indicator of hepatoblastomas. Oncogene 23: 5901-5911.
4. Hirano, W., et al. 2005. Membrane-type 1 matrix metalloproteinase cytoplasmic tail binding protein-1 (MTCBP-1) acts as an eukaryotic acireductone dioxygenase (ARD) in the methionine salvage pathway. Genes Cells 10: 565-574.
5. Gotoh, I., et al. 2007. Regulated nucleo-cytoplasmic shuttling of human acireductone dioxygenase (hADI1) and its potential role in mRNA processing. Genes Cells 12: 105-117.
6. Oram, S.W., et al. 2007. Expression and function of the human androgen-responsive gene ADI1 in prostate cancer. Neoplasia 9: 643-651.

CHROMOSOMAL LOCATION

Genetic locus: ADI1 (human) mapping to 2p25.3.

PRODUCT

ARD 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 ARD shRNA Plasmid (h): sc-72527-SH and ARD shRNA (h) Lentiviral Particles: sc-72527-V as alternate gene silencing products.

For independent verification of ARD (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-72527A, sc-72527B and sc-72527C.

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

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

ARD (H-3): sc-398325 is recommended as a control antibody for monitoring of ARD 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 ARD gene expression knockdown using RT-PCR Primer: ARD (h)-PR: sc-72527-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.