SANTA CRUZ BIOTECHNOLOGY, INC.

ARH siRNA (h): sc-106784

BACKGROUND

ARH (autosomal recessive hypercholesterolemia protein), also known as LDLRAP1 (low density lipoprotein receptor adapter protein 1), is a 308 amino acid cytoplasmic protein that contains one PID domain. ARH is an adapter protein required for efficient endocytosis of the LDL receptor (LDLR) from coated pits in polarized cells such as hepatocytes and lymphocytes. To do this, ARH acts to stabilize the interaction between the receptor and the structural components of the pits. While expressed at high levels in kidney, liver and placenta, ARH is expressed at low levels in brain, heart, muscle, colon, spleen, intestine, lung and leukocytes. Defects in the ARH gene are the cause of autosomal recessive hypercholesterolemia, a disorder caused by defective internalization of LDL receptors (LDLR) in the liver. Autosomal recessive hypercholesterolemia has the clinical features of familial hypercholesterolemia (FH), including severely elevated plasma LDL cholesterol, tuberous and tendon xanthomata, and premature atherosclerosis.

REFERENCES

1. Garcia, C.K., et al. 2001. Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. Science 292: 1394-1398.
2. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 605747. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Al-Kateb, H., et al. 2002. Mutation in the ARH gene and a chromosome 13q locus influence cholesterol levels in a new form of digenic-recessive familial hypercholesterolemia. Circ. Res. 90: 951-958.
4. Wilund, K.R., et al. 2002. Molecular mechanisms of autosomal recessive hypercholesterolemia. Hum. Mol. Genet. 11: 3019-3030.
5. He, G., et al. 2002. ARH is a modular adaptor protein that interacts with the LDL receptor, clathrin, and AP-2. J. Biol. Chem. 277: 44044-44049.
6. Mishra, S.K., et al. 2002. The autosomal recessive hypercholesterolemia (ARH) protein interfaces directly with the clathrin-coat machinery. Proc. Natl. Acad. Sci. USA 99: 16099-16104.

CHROMOSOMAL LOCATION

Genetic locus: LDLRAP1 (human) mapping to 1p36.11.

PRODUCT

ARH 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 ARH shRNA Plasmid (h): sc-106784-SH and ARH shRNA (h) Lentiviral Particles: sc-106784-V as alternate gene silencing products.

For independent verification of ARH (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-106784A, sc-106784B and sc-106784C.

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

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

ARH (F-11): sc-514106 is recommended as a control antibody for monitoring of ARH 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 ARH gene expression knockdown using RT-PCR Primer: ARH (h)-PR: sc-106784-PR (20 µl, 449 bp). 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.