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ASPH siRNA (h): sc-44989

BACKGROUND

Aspartyl/asparaginyl β -hydroxylase (ASPH) is a widely-expressed type II membrane protein involved in calcium homeostasis. Located in the endoplasmic reticulum, ASPH specifically hydroxylates an Asp or Asn residue in the epidermal growth factor-like (EGF) domains of several proteins, using iron as a cofactor. The ASPH gene encodes three proteins, ASPH, Junctin, and Junctate (or Humbug), that differ significantly in their C-terminal domains. These ASPH gene products are expressed as five transcript variants that differ by their roles in calcium storage and release, hydroxylation capabilities, and tissue specificity. While all ASPH variants are expressed in skeletal muscle, only some are detected in heart, brain, pancreas, placenta, lung, liver, and kidney tissues. In the lumen of the endoplasmic reticulum, ASPH can be processed into two different forms.

REFERENCES

1. Koriath, F., et al. 1994. Cloning and characterization of the human gene encoding aspartyl β -hydroxylase. *Gene* 150: 395-399.
2. Dinchuk, J.E., et al. 2002. Absence of post-translational aspartyl β -hydroxylation of epidermal growth factor domains in mice leads to developmental defects and an increased incidence of intestinal neoplasia. *J. Biol. Chem.* 277: 12970-12977.
3. Franzini-Armstrong, C., et al. 2005. The assembly of calcium release units in cardiac muscle. *Ann. N.Y. Acad. Sci.* 1047: 76-85.
4. Feriotto, G., et al. 2005. Myocyte enhancer factor 2 activates promoter sequences of the human β H-J-J locus, encoding aspartyl- β -hydroxylase, junctin, and junctate. *Mol. Cell. Biol.* 25: 3261-3275.
5. Kirchhefer, U., et al. 2006. Overexpression of junctin causes adaptive changes in cardiac myocyte Ca^{2+} signaling. *Cell Calcium.* 39: 131-142.

CHROMOSOMAL LOCATION

Genetic locus: ASPH (human) mapping to 8q12.3.

PRODUCT

ASPH 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 ASPH shRNA Plasmid (h): sc-44989-SH and ASPH shRNA (h) Lentiviral Particles: sc-44989-V as alternate gene silencing products.

For independent verification of ASPH (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-44989A, sc-44989B and sc-44989C.

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.

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

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

ASPH (A-10): sc-271391 is recommended as a control antibody for monitoring of ASPH 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 ASPH gene expression knockdown using RT-PCR Primer: ASPH (h)-PR: sc-44989-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.