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ASPH shRNA (h) Lentiviral Particles: sc-44989-V

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 3 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 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ASPH siRNA (h): sc-44989 and ASPH shRNA Plasmid (h): sc-44989-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

ASPH shRNA (h) Lentiviral Particles is recommended for the inhibition of ASPH expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

ASPH (F-7): sc-365012 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.