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Fatty Acid Synthase shRNA (h) Lentiviral Particles: sc-43758-V

BACKGROUND

Fatty acid biosynthesis is mediated by seven catalytic enzymes and an acyl carrier protein (ACP), to which various acyl intermediates are covalently attached. Fatty Acid Synthase (FAS) is the anabolic enzyme that contains the seven unique catalytic sites and mediates the conversion of acetyl-CoA and malonyl-CoA, in the presence of the cofactor NADPH, into long-chain saturated fatty acids, such as palmitate. Human Fatty Acid Synthase cDNA encodes a 2,504 amino acid protein. Catalytically active Fatty Acid Synthase is a homodimer. Human Fatty Acid Synthase mRNA is variably expressed with abundant levels present in brain, lung and liver. Fatty acid synthetic metabolism is abnormally elevated in tumor cells and may support cell growth or survival of malignant cancers.

REFERENCES

1. Smith, S. 1994. The animal Fatty Acid Synthase: one gene, one polypeptide, seven enzymes. *FASEB J.* 8: 1248-1259.
2. Jayakumar, A., et al. 1994. Isolation and chromosomal mapping of genomic clones encoding the human Fatty Acid Synthase gene. *Genomics* 23: 420-424.
3. Jayakumar, A., et al. 1995. Human Fatty Acid Synthase: properties and molecular cloning. *Proc. Natl. Acad. Sci. USA* 92: 8695-8699.
4. Chirala, S.S., et al. 2001. Human Fatty Acid Synthase: Role of interdomain in the formation of catalytically active synthase dimer. *Proc. Natl. Acad. Sci. USA* 98: 3104-3108.
5. Pizer, E.S., et al. 2001. Increased Fatty Acid Synthase as a therapeutic target in androgen-independent prostate cancer progression. *Prostate* 47: 102-110.

CHROMOSOMAL LOCATION

Genetic locus: FASN (human) mapping to 17q25.3.

PRODUCT

Fatty Acid Synthase 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 Fatty Acid Synthase siRNA (h): sc-43758 and Fatty Acid Synthase shRNA Plasmid (h): sc-43758-SH as alternate gene silencing products.

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.

APPLICATIONS

Fatty Acid Synthase shRNA (h) Lentiviral Particles is recommended for the inhibition of Fatty Acid Synthase 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

Fatty Acid Synthase (A-5): sc-55580 is recommended as a control antibody for monitoring of Fatty Acid Synthase 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 Fatty Acid Synthase gene expression knockdown using RT-PCR Primer: Fatty Acid Synthase (h)-PR: sc-43758-PR (20 μ l, 550 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.