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A-FABP shRNA (h) Lentiviral Particles: sc-43592-V

BACKGROUND

Fatty acid-binding proteins, designated FABPs, are a family of homologous, cytoplasmic proteins that are expressed in a highly tissue-specific manner and play an integral role in the balance between lipid and carbohydrate metabolism. FABPs mediate fatty acid (FA) and/or hydrophobic ligand uptake, transport and targeting within their respective tissues. The mechanisms underlying these actions can give rise to both passive diffusional uptake and protein-mediated transmembrane transport of FAs. FABPs are expressed in adipocytes (A-FABP), brain (B-FABP), epithelium (E-FABP, psoriasis-associated FABP, PA-FABP), striated muscle and heart (H-FABP, mammary-derived growth inhibitor or MDGI), intestine (I-FABP), liver (L-FABP), myelin (M-FABP) and testis (T-FABP). The human A-FABP gene is organized into four exons, maps to chromosome 8q21 and encodes a 132 amino acid protein. A-FABP protein comprises approximately 1% of the total cytosolic protein in human adipose tissue.

REFERENCES

1. Baxa, C.A., et al. 1989. Human adipocyte lipid-binding protein: purification of the protein and cloning of its complementary DNA. *Biochemistry* 28: 8683-8690.
2. Veerkamp, J.H. and Maatman, R.G. 1995. Cytoplasmic fatty acid-binding proteins: their structure and genes. *Prog. Lipid Res.* 34: 17-52.
3. Hotamisligil, G.S., et al. 1996. Uncoupling of obesity from Insulin resistance through a targeted mutation in AP2, the adipocyte fatty acid binding protein. *Science* 274: 1377-1379.
4. Storch, J. and Thumser, A.E. 2000. The fatty acid transport function of fatty acid-binding proteins. *Biochim. Biophys. Acta* 1486: 28-44.

CHROMOSOMAL LOCATION

Genetic locus: FABP4 (human) mapping to 8q21.13.

PRODUCT

A-FABP 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 A-FABP siRNA (h): sc-43592 and A-FABP shRNA Plasmid (h): sc-43592-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.

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.

APPLICATIONS

A-FABP shRNA (h) Lentiviral Particles is recommended for the inhibition of A-FABP 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 lentiviral transducing particles per milliliter; contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

A-FABP (B-4): sc-271529 is recommended as a control antibody for monitoring of A-FABP 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 A-FABP gene expression knockdown using RT-PCR Primer: A-FABP (h)-PR: sc-43592-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.

PROTOCOLS

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