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PPAR α shRNA (h2) Lentiviral Particles: sc-44323-V

BACKGROUND

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that can be activated by a variety of compounds including fibrates, thiazolidinediones, prostaglandins and fatty acids. Three PPAR subtypes, designated PPAR α , PPAR β (also designated PPAR δ) and PPAR γ , have been described. PPARs promote transcription by forming heterodimers with members of the retinoid X receptor (RXR) family of steroid receptors and binding to specific DNA motifs termed PPAR-response elements (PPREs). PPAR α is abundant in primary hepatocytes where it regulates the expression of proteins involved in fatty acid metabolism. PPAR β is the most widely distributed subtype and is often expressed at high levels. PPAR γ is predominantly seen in adipose tissue where it plays a critical role in regulating adipocyte differentiation. Interestingly, both the orphan nuclear hormone receptor LXR α and thyroid receptor (TR) have been shown to act as antagonists of PPAR α /RXR α binding to PPREs.

REFERENCES

1. Brun, R.P., et al. 1996. Differential activation of adipogenesis by multiple PPAR isoforms. *Genes Dev.* 10: 974-984.
2. Mansen, A., et al. 1996. Expression of the peroxisome proliferator-activated receptor (PPAR) in the mouse colonic mucosa. *Biochem. Biophys. Res. Commun.* 222: 844-851.
3. Lemberger, T., et al. 1996. Expression of the peroxisome proliferator-activated receptor α gene is stimulated by stress and follows a diurnal rhythm. *J. Biol. Chem.* 271: 1764-1769.
4. Braissant, O., et al. 1996. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR α , β , and γ in the adult rat. *Endocrinology* 137: 354-366.

CHROMOSOMAL LOCATION

Genetic locus: PPARA (human) mapping to 22q13.31.

PRODUCT

PPAR α shRNA (h2) 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 PPAR α siRNA (h2): sc-44323 and PPAR α shRNA Plasmid (h2): sc-44323-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

PPAR α shRNA (h2) Lentiviral Particles is recommended for the inhibition of PPAR α 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

PPAR α (H-2): sc-398394 is recommended as a control antibody for monitoring of PPAR α 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 PPAR α gene expression knockdown using RT-PCR Primer: PPAR α (h2)-PR: sc-44323-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.