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# PPAR $\gamma$ shRNA (h2) Lentiviral Particles: sc-44220-V

## BACKGROUND

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor subfamily of transcription factors. PPARs form heterodimers with retinoid X receptors (RXRs). These heterodimers regulate transcription of genes involved in Insulin action, adipocyte differentiation, lipid metabolism and inflammation. PPAR $\gamma$  is implicated in numerous diseases including obesity, diabetes, atherosclerosis and cancer. PPAR $\gamma$  activators include prostanoids, fatty acids, thiazolidinediones and N-(2-benzoylphenyl) tyrosine analogues. A key component in adipocyte differentiation and fat-specific gene expression, PPAR $\gamma$  may modulate macrophage functions such as proinflammatory activities, and stimulate oxidized low-density lipoprotein (x-LDL) uptake. A Pro12Ala polymorphism of the PPAR $\gamma$ 2 gene has been reported to reduce transactivation activity *in vitro*. This substitution may affect the immune response to ox-LDL and be associated with type 2 diabetes. In addition, the Pro12Ala variant of the PPAR $\gamma$ 2 gene maybe correlated with abdominal obesity in type 2 diabetes.

## 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  $\alpha$  gene is stimulated by stress and follows a diurnal rhythm. *J. Biol. Chem.* 271: 1764-1769.
4. Miyata, K.S., et al. 1996. The orphan nuclear hormone receptor LXR  $\alpha$  interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling. *J. Biol. Chem.* 271: 9189-9192.
5. Braissant, O., et al. 1996. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR- $\alpha$ , - $\beta$ , and - $\gamma$  in the adult rat. *Endocrinology* 137: 354-366.

## CHROMOSOMAL LOCATION

Genetic locus: PPARG (human) mapping to 3p25.2.

## PRODUCT

PPAR $\gamma$  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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 $\gamma$  siRNA (h2): sc-44220 and PPAR $\gamma$  shRNA Plasmid (h2): sc-44220-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

PPAR $\gamma$  shRNA (h2) Lentiviral Particles is recommended for the inhibition of PPAR $\gamma$  expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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 $\gamma$  (B-5): sc-271392 is recommended as a control antibody for monitoring of PPAR $\gamma$  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<sup>™</sup> compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker<sup>™</sup> 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<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PPAR $\gamma$  gene expression knockdown using RT-PCR Primer: PPAR $\gamma$  (h2)-PR: sc-44220-PR (20  $\mu$ l, 446 bp). 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.

## PROTOCOLS

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