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- Mindermengenzuschlag
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SHP shRNA (h) Lentiviral Particles: sc-44101-V

BACKGROUND

SHP (short heterodimer partner and small heterodimer partner) is an orphan nuclear receptor containing the dimerization and ligand-binding domains found in other nuclear receptors, but lacking the conserved DNA binding domain. SHP is specifically expressed in liver and other tissues, including fetal liver and adrenal gland, as well as adult spleen and small intestine. In addition, SHP is highly expressed in the murine macrophage cell line RAW 264.7 but suppressed by oxLDL and 13-HODE, which is a ligand for PPAR γ . SHP interacts with nuclear receptors, including thyroid receptor, retinoic acid receptors (RAR and RXR) and estrogen receptors (ER α and ER β). SHP functions as a negative regulator of these receptors by at least three mechanisms: inhibition of DNA binding via dimerization, direct antagonism of coactivator function through competition and possibly transrepression via recruitment of putative corepressors. In oxLDL-treated, resting macrophage cells, SHP acts as a transcription coactivator of NF κ B, suggesting that SHP is a modulatory component in the regulation of the transcriptional activities of NF κ B. Lastly, negative feedback regulation of a hepatic bile acid transporter, Ntcp, is controlled by bile acid-activated FXR via induction of SHP to protect the hepatocyte from bile acid-mediated damage in cholestatic conditions.

REFERENCES

1. Seol, W., et al. 1996. An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. *Science* 272: 1336-1339.
2. Lee, H.K., et al. 1998. Structure and expression of the orphan nuclear receptor SHP gene. *J. Biol. Chem.* 273: 14398-14402.
3. Seol, W., et al. 1998. Inhibition of estrogen receptor action by the orphan receptor SHP (short heterodimer partner). *Mol. Endocrinol.* 12: 1551-1557.

CHROMOSOMAL LOCATION

Genetic locus: NROB2 (human) mapping to 1p36.11.

PRODUCT

SHP 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 SHP siRNA (h): sc-44101 and SHP shRNA Plasmid (h): sc-44101-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

SHP shRNA (h) Lentiviral Particles is recommended for the inhibition of SHP 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

SHP (F-12): sc-271469 is recommended as a control antibody for monitoring of SHP 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 SHP gene expression knockdown using RT-PCR Primer: SHP (h)-PR: sc-44101-PR (20 μ l, 437 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.