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3 β -HSD shRNA (m) Lentiviral Particles: sc-44470-V

BACKGROUND

3 β -hydroxysteroid dehydrogenase (3 β -HSD), also known as HSD3B1 or HSD3B3, is a bifunctional enzyme that plays a crucial role in the synthesis of all classes of hormonal steroids. Two human 3 β -HSD proteins, designated type I (3 β -HSD) and type II (3 β -HSD2), are expressed by different genes and function in different areas of the body. Localized to the membrane of the endoplasmic reticulum (ER) and expressed in skin and placenta, 3 β -HSD is the type I protein that catalyzes the oxidative conversion of δ^5 -ene-3- β -hydroxy steroid, as well as the conversion of various ketosteroids. Defects in the gene encoding 3 β -HSD are associated with classic salt wasting, genital ambiguity, hypogonadism, Insulin-resistant polycystic ovary syndrome (PCOS) and an increased susceptibility to prostate cancer. Additionally, congenital deficiency of 3 β -HSD activity results in a severe depletion of steroid formation which can be lethal in young children.

REFERENCES

1. Thomas, J.L., et al. 2002. Structure/function relationships responsible for the kinetic differences between human type 1 and type 2 3 β -hydroxysteroid dehydrogenase and for the catalysis of the type 1 activity. *J. Biol. Chem.* 277: 42795-42801.
2. Thomas, J.L., et al. 2003. Structure/function relationships responsible for coenzyme specificity and the isomerase activity of human type 1 3 β -hydroxysteroid dehydrogenase/isomerase. *J. Biol. Chem.* 278: 35483-35490.
3. Foti, D.M., et al. 2004. YY1 binding within the human HSD3B2 gene intron 1 is required for maximal basal promoter activity: identification of YY1 as the 3 β 1-A factor. *J. Mol. Endocrinol.* 33: 99-9119.
4. Thomas, J.L., et al. 2004. Serine 124 completes the Tyr, Lys and Ser triad responsible for the catalysis of human type 1 3 β -hydroxysteroid dehydrogenase. *J. Mol. Endocrinol.* 33: 253-261.

CHROMOSOMAL LOCATION

Genetic locus: Hsd3b1 (mouse) mapping to 3 F2.2.

PRODUCT

3 β -HSD shRNA (m) 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 3 β -HSD siRNA (m): sc-44470 and 3 β -HSD shRNA Plasmid (m): sc-44470-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

3 β -HSD shRNA (m) Lentiviral Particles is recommended for the inhibition of 3 β -HSD expression in mouse 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

3 β -HSD (C-18): sc-30821 is recommended as a control antibody for monitoring of 3 β -HSD 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor 3 β -HSD gene expression knockdown using RT-PCR Primer: 3 β -HSD (m)-PR: sc-44470-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.