

Produktinformation



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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien T. +43(0)1 489 3961-0 F. +43(0)1 489 3961-7 <u>mail@szabo-scandic.com</u> www.szabo-scandic.com

SANTA CRUZ BIOTECHNOLOGY, INC.

3β-HSD shRNA (h) Lentiviral Particles: sc-43584-V



BACKGROUND

3β-hydroxysteroid dehydrogenase (3β-HSD), also known as HSD3B1 or HSDB3, is a bifunctional enzyme that plays a crucial role in the synthesis of all classes of hormonal steroids. Two human 3β-HSD proteins, designated type I (3β -HSD) and type II (3β -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, 3β-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 3β -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 3β-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 3β-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 3β-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 3β1-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 (human) mapping to 1p12.

PRODUCT

3β-HSD 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 µl frozen stock containing 1.0 x 10⁶ 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 3β -HSD siRNA (h): sc-43584 and 3β-HSD shRNA Plasmid (h): sc-43584-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

 3β -HSD shRNA (h) Lentiviral Particles is recommended for the inhibition of 3β-HSD expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

3B-HSD (C-18): sc-30821 is recommended as a control antibody for monitoring of 3β-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 3β-HSD gene expression knockdown using RT-PCR Primer: 3β-HSD (h)-PR: sc-43584-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.