

3 β -HSD siRNA (h): sc-43584

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: 99-119.
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.
5. Carbanaru, G., et al. 2004. The hormonal phenotype of Nonclassic 3 β -hydroxysteroid dehydrogenase (HSD3B) deficiency in hyperandrogenic females is associated with insulin-resistant polycystic ovary syndrome and is not a variant of inherited HSD3B2 deficiency. *J. Clin. Endocrinol. Metab.* 89: 783-794.

CHROMOSOMAL LOCATION

Genetic locus: HSD3B1 (human) mapping to 1p12.

PRODUCT

3 β -HSD siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see 3 β -HSD shRNA Plasmid (h): sc-43584-SH and 3 β -HSD shRNA (h) Lentiviral Particles: sc-43584-V as alternate gene silencing products.

For independent verification of 3 β -HSD (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43584A, sc-43584B and sc-43584C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

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

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

3 β -HSD (A-1): sc-515120 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

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 (h)-PR: sc-43584-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.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.