SANTA CRUZ BIOTECHNOLOGY, INC.

TSHβ siRNA (h): sc-39321

BACKGROUND

Various hormones are secreted from the anterior pituitary during development and growth, including thyroid-stimulating hormone (TSH, also known as thyrotropin), follicle-stimulating hormone (FSH) and leutinizing hormone (LH). TSH, FSH, and LH are heterodimers formed from a common α chain and a unique β chain. TSH is a glycoprotein involved in the control of thyroid structure and metabolism, which stimulates the release of the thyroid hormones. TSHβ is regulated by thyroid hormone (T3) and various retinoid compounds. TSHβ binds to the thyroid-stimulating hormone receptor (TSHR), which plays a major role in regulating thyroid function. TSHR is thought to exist in multiple glycosylation states. The third cytoplasmic loop of TSHR has been identified as critical for its role in regulating inositol phosphate and cAMP formation.

REFERENCES

1. Kosugi, S., et al. 1993. Substitutions of different regions of the third cytoplasmic loop of the thyrotropin (TSH) receptor have selective effects on constitutive, TSH-, and TSH receptor autoantibody-stimulated phosphoinositide and 3',5'-cyclic adenosine monophosphate signal generation. Mol. Endocrinol. 7: 1009-1020.
2. Graves, P.N., et al. 1996. Multimeric complex formation by the thyrotropin receptor in solubilized thyroid membranes. Endocrinology 137: 3915-3920.
3. Sanders, J., et al. 1997. Understanding the thyrotropin receptor function-structure relationship. Baillieres Clin. Endocrinol. Metab. 11: 451-479.
4. Breen, J.J., et al. 1997. The rat TSHβ gene contains distinct response elements for regulation by retinoids and thyroid hormone. Mol. Cell. Endocrinol. 131: 137-146.
5. Moyle, W.R., et al. 1998. Functional homodimeric glycoprotein hormones: implications for hormone action and evolution. Chem. Biol. 5: 241-254.
6. Sasaki, S., et al. 1999. Ligand-induced recruitment of a histone deacetylase in the negative-feedback regulation of the thyrotropin β gene. EMBO J. 18: 5389-5398.
7. Okada, R., et al. 2000. Cloning of bullfrog thyroid-stimulating hormone (TSH) β subunit cDNA: expression of TSHβ mRNA during metamorphosis. Gen. Comp. Endocrinol. 119: 224-231.

CHROMOSOMAL LOCATION

Genetic locus: TSHB (human) mapping to 1p13.2.

PRODUCT

TSHβ 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TSHβ shRNA Plasmid (h): sc-39321-SH and TSHβ shRNA (h) Lentiviral Particles: sc-39321-V as alternate gene silencing products.

For independent verification of TSHβ (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-39321A, sc-39321B and sc-39321C.

STORAGE AND RESUSPENSION

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

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

APPLICATIONS

TSHβ siRNA (h) is recommended for the inhibition of TSHβ 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 µM in 66 µl. 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

TSHβ (D-6): sc-365801 is recommended as a control antibody for monitoring of TSHβ 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 TSHβ gene expression knockdown using RT-PCR Primer: TSHβ (h)-PR: sc-39321-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.