SANTA CRUZ BIOTECHNOLOGY, INC.

TRα siRNA (m): sc-36708

BACKGROUND

Thyroid hormone nuclear receptors (TRs) are ligand-dependent transcription factors which regulate growth, differentiation and development and represent members of the steroid/retinoic acid superfamily. The two genes encoding TRs identified to date, TRα and TRβ, have been mapped to human chromosomes 17q21.1 and 3p24.2, respectively. TRs bind to thyroid hormone response elements (TREs) with half-site binding motifs in the orientation of palindromes, direct repeats or inverted palindromes. The affinities of binding are both variable and influenced differentially by 3,5,3′-triiodo-L-thyronine (T3). Transcriptional regulation by TRs is also modulated by heterodimerization with TR nuclear accessory proteins, the most extensively characterized of which are the retinoid X receptors (RXRα, RXRβ and RXRγ). To a certain extent, this activity is regulated by differential phosphorylation of TRs. Thus, not only are the biological activities of TRs regulated by heterodimerization with RXRs, but in addition, the gene regulatory activities of TRs are linked to other hormonal pathways. TRα1 can display both a nuclear and undefined cytoplasmic location, and is the only TR that is imported into the mitochondrial matrix.

REFERENCES

1. Näär, A., et al. 1991. The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. Cell 65: 1267-1271.
2. Lazar, M.A. 1993. Thyroid hormone receptors: multiple forms, multiple possibilities. Endocrinol. Rev. 14: 184-193.
3. Meier, C.A., et al. 1993. Interaction of human TRβ1 and its mutants with DNA and RXRβ. T3 response element-dependent dominant negative potency. J. Clin. Invest. 92: 1986-1993.

CHROMOSOMAL LOCATION

Genetic locus: Thra (mouse) mapping to 11 D.

PRODUCT

TRα siRNA (m) 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 TRα shRNA Plasmid (m): sc-36708-SH and TRα shRNA (m) Lentiviral Particles: sc-36708-V as alternate gene silencing products.

For independent verification of TRα (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36708A, sc-36708B and sc-36708C.

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

TRα siRNA (m) is recommended for the inhibition of TRα expression in mouse 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

TRα1/β1 (C1): sc-739 is recommended as a control antibody for monitoring of TRα 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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) 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 TRα gene expression knockdown using RT-PCR Primer: TRα (m)-PR: sc-36708-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kassotis, C.D., et al. 2019. Thyroid receptor antagonism as a contributory mechanism for adipogenesis induced by environmental mixtures in 3T3-L1 cells. Sci. Total Environ. 666: 431-444.

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.