

TRAP95 siRNA (h): sc-38587

BACKGROUND

In mammalian cells, transcription is regulated in part by high molecular weight coactivating complexes that mediate signaling between transcriptional activators and initiation factors. These complexes include the thyroid hormone receptor-associated protein (TRAP) complex, which interacts with thyroid receptors (TR), vitamin D receptors and other steroid receptors to facilitate hormone induced transcriptional activation. The TRAP complex consists of numerous proteins ranging in size including TRAP95, TRAP100, TRAP150, TRAP220 and TRAP230, that are characterized by the presence of a nuclear receptor recognition motif which mediates the ligand-dependent binding of TRAP proteins to the nuclear receptors. TRAP220 and TRAP100 are widely expressed and most abundantly detected in skeletal muscle, heart and placenta. TRAP95, TRAP150 and TRAP230 facilitate TR induced transcription by associating with an additional transcriptional coactivating complex SMCC (SRB and MED protein cofactor complex), which consists of various subunits that share homology with several components of the yeast transcriptional mediator complexes.

REFERENCES

1. Yuan, C.X., et al. 1998. The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. *Proc. Natl. Acad. Sci. USA* 95: 7939-7944.
2. Jiang, Y.W., et al. 1998. Mammalian mediator of transcriptional regulation and its possible role as an end-point of signal transduction pathways. *Proc. Natl. Acad. Sci. USA* 95: 8538-8543.
3. Zhang, J. and Fondell, J.D. 1999. Identification of mouse TRAP100: a transcriptional coregulatory factor for thyroid hormone and vitamin D receptors. *Mol. Endocrinol.* 13: 1130-1140.
4. Treuter, E., et al. 1999. Competition between thyroid hormone receptor-associated protein (TRAP) 220 and transcriptional intermediary factor (TIF) 2 for binding to nuclear receptors. Implications for the recruitment of TRAP and p160 coactivator complexes. *J. Biol. Chem.* 274: 6667-6677.
5. Kumar, R. and Thompson, E.B. 1999. The structure of the nuclear hormone receptors. *Steroids* 64: 310-319.

CHROMOSOMAL LOCATION

Genetic locus: MED16 (human) mapping to 19p13.3.

PRODUCT

TRAP95 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 TRAP95 shRNA Plasmid (h): sc-38587-SH and TRAP95 shRNA (h) Lentiviral Particles: sc-38587-V as alternate gene silencing products.

For independent verification of TRAP95 (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-38587A, sc-38587B and sc-38587C.

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 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

TRAP95 siRNA (h) is recommended for the inhibition of TRAP95 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

TRAP95 (F83): sc-130439 is recommended as a control antibody for monitoring of TRAP95 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 TRAP95 gene expression knockdown using RT-PCR Primer: TRAP95 (h)-PR: sc-38587-PR (20 μ l). 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.