

Med25 siRNA (h): sc-97106

BACKGROUND

In mammalian cells, transcription is regulated in part by high molecular weight coactivating complexes that mediate signals between transcriptional activators and RNA polymerase II (Pol II). The mediator complex is one such multi-protein structure that functions as a bridge between regulatory proteins and Pol II, thereby regulating Pol II-dependent transcription. Med25 (mediator complex subunit 25), also known as P78, ACID1, ARC92 or PTOV2, is a 747 amino acid nuclear protein that functions as a component of the mediator complex. Expressed ubiquitously with highest levels in spleen, heart, brain, kidney, placenta and skeletal muscle, Med25 is one of several proteins that work in tandem and serve as a scaffold for the assembly of a functional preinitiation complex with Pol II and general transcription factors. Five isoforms of Med25 exist as a result of alternative splicing events.

REFERENCES

1. Näär, A.M., et al. 1999. Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature* 398: 828-832.
2. Wang, C., et al. 2002. A prostate-derived cDNA that is mapped to human chromosome 19 encodes a novel protein. *Biochem. Biophys. Res. Commun.* 296: 281-287.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610197. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Sato, S., et al. 2003. A mammalian homolog of *Drosophila melanogaster* transcriptional coactivator intersex is a subunit of the mammalian Mediator complex. *J. Biol. Chem.* 278: 49671-49674.

CHROMOSOMAL LOCATION

Genetic locus: MED25 (human) mapping to 19q13.33.

PRODUCT

Med25 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 Med25 shRNA Plasmid (h): sc-97106-SH and Med25 shRNA (h) Lentiviral Particles: sc-97106-V as alternate gene silencing products.

For independent verification of Med25 (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-97106A, sc-97106B and sc-97106C.

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

Med25 siRNA (h) is recommended for the inhibition of Med25 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

Med25 (A-7): sc-393759 is recommended as a control antibody for monitoring of Med25 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 Med25 gene expression knockdown using RT-PCR Primer: Med25 (h)-PR: sc-97106-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Shi, Z., et al. 2014. Med25 is required for estrogen receptor α (ER α)-mediated regulation of human CYP2C9 expression. *Biochem. Pharmacol.* 90: 425-431.

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.