

Med8 siRNA (m): sc-149359

BACKGROUND

In mammalian cells, transcription is regulated in part by high molecular weight co-activating 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. Med8 (Mediator complex subunit 8), also known as arc32 (activator-recruited cofactor 32 kDa component), is a 268 amino acid protein that localizes to the nucleus and exists as a component of the mediator complex. Involved in the pathway of protein modification and ubiquitination, Med8 is involved in transcriptional regulation and may also recruit E3 ubiquitin-protein ligase complexes to proteins targeted for proteasomal degradation. Multiple isoforms of Med8 exist due to alternative splicing events.

REFERENCES

1. 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.
2. Näär, A.M., et al. 1999. Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature* 398: 828-832.
3. Brower, C.S., et al. 2002. Mammalian mediator subunit mMed8 is an Elongin BC-interacting protein that can assemble with Cul2 and Rbx1 to reconstitute a ubiquitin ligase. *Proc. Natl. Acad. Sci. USA* 99: 10353-10358.
4. Sato, S., et al. 2003. Identification of mammalian mediator subunits with similarities to yeast mediator subunits Srb5, Srb6, Med11, and Rox3. *J. Biol. Chem.* 278: 15123-15127.
5. 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.
6. Tomomori-Sato, C., et al. 2004. A mammalian mediator subunit that shares properties with *Saccharomyces cerevisiae* mediator subunit Cse2. *J. Biol. Chem.* 279: 5846-5851.

CHROMOSOMAL LOCATION

Genetic locus: Med8 (mouse) mapping to 4 D2.1.

PRODUCT

Med8 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Med8 shRNA Plasmid (m): sc-149359-SH and Med8 shRNA (m) Lentiviral Particles: sc-149359-V as alternate gene silencing products.

For independent verification of Med8 (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-149359A, sc-149359B and sc-149359C.

RESEARCH USE

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

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

Med8 siRNA (m) is recommended for the inhibition of Med8 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 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

Med8 (A-5): sc-365960 is recommended as a control antibody for monitoring of Med8 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 Med8 gene expression knockdown using RT-PCR Primer: Med8 (m)-PR: sc-149359-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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