

Med10 siRNA (h): sc-91760

BACKGROUND

The mediator complex is a multiprotein coactivator that is involved in the regulated transcription of Pol II-dependent genes. Functioning as a bridge to convey information from gene-specific regulatory proteins to the basal Pol II transcription machinery, the mediator complex is recruited to promoter regions by directly interacting with regulatory proteins. The mediator complex also serves as a scaffold for the assembly of a functional pre-initiation complex with Pol II and other general transcription factors. Med10 (Mediator of RNA polymerase II transcription subunit 10), also designated Transformation-related gene 17 protein, is a 135 amino acid nuclear protein that is a middle domain component of the mediator complex. Reduction of Med10 protein levels within cells leads to an enhancement of Wnt and Nodal signaling pathways. Also, in yeast, mutation the gene encoding Med10, as well as other middle domain mediator components, leads to enhanced transcription wild-type heat-shock genes.

REFERENCES

1. Gu, W., et al. 1999. A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation. *Mol. Cell* 3: 97-108.
2. Malik, S., et al. 2000. The USA-derived transcriptional coactivator PC2 is a submodule of TRAP/SMCC and acts synergistically with other PCs. *Mol. Cell* 5: 753-760.
3. Akoulitchev, S., et al. 2000. TFIH is negatively regulated by cdk8-containing mediator complexes. *Nature* 407: 102-106.
4. Sato, S., et al. 2004. A set of consensus mammalian mediator subunits identified by multidimensional protein identification technology. *Mol. Cell* 14: 685-691.
5. Petroziello, J., et al. 2004. Suppression subtractive hybridization and expression profiling identifies a unique set of genes overexpressed in non-small-cell lung cancer. *Oncogene* 23: 7734-7745.
6. Zhang, X., et al. 2005. MED1/TRAP220 exists predominantly in a TRAP/Mediator subpopulation enriched in RNA polymerase II and is required for ER-mediated transcription. *Mol. Cell* 19: 89-100.
7. Singh, H., et al. 2006. A functional module of yeast mediator that governs the dynamic range of heat-shock gene expression. *Genetics* 172: 2169-2184.

CHROMOSOMAL LOCATION

Genetic locus: MED10 (human) mapping to 5p15.31.

PRODUCT

Med10 siRNA (h) is a pool of 2 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 Med10 shRNA Plasmid (h): sc-91760-SH and Med10 shRNA (h) Lentiviral Particles: sc-91760-V as alternate gene silencing products.

For independent verification of Med10 (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-91760A and sc-91760B.

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

Med10 siRNA (h) is recommended for the inhibition of Med10 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

Med10 (C-2): sc-393450 is recommended as a control antibody for monitoring of Med10 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 Med10 gene expression knockdown using RT-PCR Primer: Med10 (h)-PR: sc-91760-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.