

Med6 siRNA (h): sc-38580

BACKGROUND

In mammalian cells, transcription is regulated in part by high molecular weight coactivating complexes that mediate signals between transcriptional activators and RNA polymerase. These complexes include the SMCC (SRB and MED protein cofactor complex), which consists of various subunits that share homology with several components of the yeast transcriptional mediator complexes, and include the human proteins Srb7, Med6 (also designated DRIP33) and Med7 (also designated DRIP34). SMCC associates with the RNAPII (RNA polymerase II) holoenzyme through Srb7 and, in turn, enhances gene-specific activation or repression induced by DNA-binding transcription factors. Med6 and Med7, as well as other components of SMCC, associate with coactivator proteins from the TRAP (thyroid hormone receptor-activating protein) complex and DRIP (for vitamin D receptor interacting protein) complex to facilitate steroid receptor dependent transcriptional activation. Additionally, SMCC associates with PC4 (positive cofactor 4) to repress basal transcription independent of RNAPII activity.

REFERENCES

1. Malik, S., et al. 1998. A dynamic model for PC4 coactivator function in RNA polymerase II transcription. *Proc. Natl. Acad. Sci. USA* 95: 2192-2197.
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. Gu, W., et al. 1999. A novel human Srb/Med-containing cofactor complex, SMCC, involved in transcription regulation. *Mol. Cell* 3: 97-108.
4. Xiao, H., et al. 1999. The human homologue of *Drosophila* TRF-proximal protein is associated with an RNA polymerase II-Srb complex. *J. Biol. Chem.* 274: 3937-3940.
5. Ito, M., et al. 1999. Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. *Mol. Cell* 3: 361-370.
6. Rachez, C., et al. 1999. Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. *Nature* 398: 824-828.

CHROMOSOMAL LOCATION

Genetic locus: MED6 (human) mapping to 14q24.2.

PRODUCT

Med6 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 Med6 shRNA Plasmid (h): sc-38580-SH and Med6 shRNA (h) Lentiviral Particles: sc-38580-V as alternate gene silencing products.

For independent verification of Med6 (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-38580A, sc-38580B and sc-38580C.

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

Med6 siRNA (h) is recommended for the inhibition of Med6 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

Med6 (D-2): sc-390474 is recommended as a control antibody for monitoring of Med6 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 MarkerTM 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 Med6 gene expression knockdown using RT-PCR Primer: Med6 (h)-PR: sc-38580-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.