

Med29 siRNA (h): sc-97527

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. Med29 (mediator complex subunit 29), also known as IXL (intersex-like), is a 200 amino acid nuclear protein and component of the mediator complex. Widely expressed in embryo and adult tissue, Med29 is considered a novel amplification target gene in pancreatic cancer and is encoded by a gene that maps to human chromosome 19q13.2. Chromosome 19 consists of over 63 million bases, houses approximately 1,400 genes and is recognized for having the greatest gene density of the human chromosomes.

REFERENCES

1. 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.
2. Wang, Y., et al. 2004. IXL, a new subunit of the mammalian Mediator complex, functions as a transcriptional suppressor. *Biochem. Biophys. Res. Commun.* 325: 1330-1338.
3. 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.
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. 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.
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CHROMOSOMAL LOCATION

Genetic locus: MED29 (human) mapping to 19q13.2.

PRODUCT

Med29 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 Med29 shRNA Plasmid (h): sc-97527-SH and Med29 shRNA (h) Lentiviral Particles: sc-97527-V as alternate gene silencing products.

For independent verification of Med29 (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-97527A, sc-97527B and sc-97527C.

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

Med29 siRNA (h) is recommended for the inhibition of Med29 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

Med29 (B-1): sc-393800 is recommended as a control antibody for monitoring of Med29 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 Med29 gene expression knockdown using RT-PCR Primer: Med29 (h)-PR: sc-97527-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.