

CRSP70 siRNA (m): sc-38574

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 CRSP (for cofactor required for Sp1 activation), which is required, in conjunction with TAFII, for transcriptional activation by Sp1. CRSP is ubiquitously expressed in various tissues and functions as a multimeric complex that consists of nine distinct subunits. Several members of the CRSP family share sequence similarity with multiple components of the yeast transcriptional mediator proteins, including CRSP150, which is related to yeast Rgr1, and CRSP70, which is similar to the elongation factor TFIIS. CRSP77 and CRSP150 are also related to proteins within the putative murine mediator complex, while CRSP130 and CRSP34 are largely unrelated to either murine or yeast proteins. CRSP subunits also associate with larger multimeric co-activator complexes, including ARC/DRI, which binds directly to SREBP and nuclear hormone receptors to facilitate transcription, and with NAT, a polymerase II-interacting complex that represses activated transcription.

REFERENCES

1. Kim, Y.J., et al. 1994. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell* 77: 599-608.
2. Myers, L.C., et al. 1998. The Med proteins of yeast and their function through the RNA polymerase II carboxy-terminal domain. *Genes Dev.* 12: 45-54.
3. 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.
4. Ryu, S., et al. 1999. The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. *Nature* 397: 446-450.
5. Ryu, S., et al. 1999. Purification of transcription cofactor complex CRSP. *Proc. Natl. Acad. Sci. USA* 96: 7137-7142.
6. Andel, F., 3rd., et al. 1999. Three-dimensional structure of the human TFIID-IIA-IIB complex. *Science* 286: 2153-2156.

CHROMOSOMAL LOCATION

Genetic locus: Med26 (mouse) mapping to 8 B3.3.

PRODUCT

CRSP70 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 CRSP70 shRNA Plasmid (m): sc-38574-SH and CRSP70 shRNA (m) Lentiviral Particles: sc-38574-V as alternate gene silencing products.

For independent verification of CRSP70 (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-38574A, sc-38574B and sc-38574C.

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

CRSP70 siRNA (m) is recommended for the inhibition of CRSP70 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

CRSP70 (G-11): sc-166614 is recommended as a control antibody for monitoring of CRSP70 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 CRSP70 gene expression knockdown using RT-PCR Primer: CRSP70 (m)-PR: sc-38574-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.