

Mi2-β siRNA (h): sc-37953

BACKGROUND

In the intact cell, DNA closely associates with histones and other nuclear proteins to form chromatin. The remodeling of chromatin is believed to be a critical component of transcriptional regulation and a major source of this remodeling is brought about by the acetylation of nucleosomal histones. Acetylation of lysine residues in the amino terminal tail domain of histone results in an allosteric change in the nucleosomal conformation and an increased accessibility to transcription factors by DNA. Conversely, the deacetylation of histones is associated with transcriptional silencing. Chromatin structure alteration may be brought about by the action of ATP-dependent multiprotein complexes. One such complex is the mSin3 corepressor complex, which contains mSin3, the histone deacetylases HDAC1 and HDAC2, the associated proteins SAP 30 and SAP 18, and the autoantigens Mi2-α and Mi2-β.

REFERENCES

1. Lee, D.Y., et al. 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell* 72: 73-82.
2. Braunstein, M., et al. 1993. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes Dev.* 7: 592-604.
3. Bauer, W.R., et al. 1994. Nucleosome structural changes due to acetylation. *J. Mol. Biol.* 236: 685-690.
4. Seelig, H.P., et al. 1995. The major dermatomyositis-specific Mi-2 autoantigen is a presumed helicase involved in transcriptional activation. *Arthritis Rheum.* 38: 1389-1399.
5. Kingston, R.E., et al. 1996. Repression and activation by multiprotein complexes that alter chromatin structure. *Genes Dev.* 10: 905-920.

CHROMOSOMAL LOCATION

Genetic locus: CHD4 (human) mapping to 12p13.31.

PRODUCT

Mi2-β 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Mi2-β shRNA Plasmid (h): sc-37953-SH and Mi2-β shRNA (h) Lentiviral Particles: sc-37953-V as alternate gene silencing products.

For independent verification of Mi2-β (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-37953A, sc-37953B and sc-37953C.

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 μl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μl of RNase-free water makes a 10 μM solution in a 10 μM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Mi2-β siRNA (h) is recommended for the inhibition of Mi2-β 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 μM in 66 μl. 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

Mi2-β (F-7): sc-365639 is recommended as a control antibody for monitoring of Mi2-β 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Mi2-β gene expression knockdown using RT-PCR Primer: Mi2-β (h)-PR: sc-37953-PR (20 μl, 556 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Velichko, A.K., et al. 2019. Hypoosmotic stress induces R loop formation in nucleoli and ATR/ATM-dependent silencing of nucleolar transcription. *Nucleic Acids Res.* E-published.

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.