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SSX1 siRNA (h): sc-44120

BACKGROUND

The transcriptional coactivator SYT (synovial translocation protein) contains a conserved amino terminal SNH domain and a carboxy terminal QPGY domain, which is a functioning transcriptional activating sequence. Synovial sarcoma translocation (SSX) proteins, including SSX1-5, are transcriptional repressors that contain a repressor domain in their carboxy termini. SSX proteins are localized to the nucleus and expressed in testis and several types of cancers and, therefore, they are classified as C/T (cancer/testis) antigens. The t(x;18) translocation results in the fusion of the amino terminus of SYT to the carboxy terminus of either SSX1 or SSX2; both fusions result in the production of transcriptional activators. SSX1 (synovial sarcoma, X breakpoint 1), also known as SSRC or CT5.1 (cancer/testis antigen 5.1), is a 188 amino acid protein that is implicated in transcription modulation and belongs to the SSX family. Highly expressed in testis, SSX1 is also found in rhabdomyosarcoma and fibrosarcoma cell lines, but is expressed only at low levels in thyroid.

REFERENCES

- Clark, J., et al. 1994. Identification of novel genes, SYT and SSX, involved in the t(X;18)(p11.2;q11.2) translocation found in human synovial sarcoma. *Nat. Genet.* 7: 502-508.
- Crew, A.J., et al. 1995. Fusion of SYT to two genes, SSX1 and SSX2, encoding proteins with homology to the Krüppel-associated box in human synovial sarcoma. *EMBO J.* 14: 2333-2340.
- Chand, A., et al. 1995. Long-range organization of reiterated sequences, including the SSX1 cDNA at the OATL1 cluster in Xp11.23. *Genomics* 30: 545-552.
- de Leeuw, B., et al. 1995. Identification of two alternative fusion genes, SYT-SSX1 and SYT-SSX2, in t(X;18)(p11.2;q11.2)-positive synovial sarcomas. *Hum. Mol. Genet.* 4: 1097-1099.
- Kawai, A., et al. 1998. SYT-SSX gene fusion as a determinant of morphology and prognosis in synovial sarcoma. *N. Engl. J. Med.* 338: 153-160.

CHROMOSOMAL LOCATION

Genetic locus: SSX1 (human) mapping to Xp11.23.

PRODUCT

SSX1 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 SSX1 shRNA Plasmid (h): sc-44120-SH and SSX1 shRNA (h) Lentiviral Particles: sc-44120-V as alternate gene silencing products.

For independent verification of SSX1 (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-44120A and sc-44120B.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

SSX1 siRNA (h) is recommended for the inhibition of SSX1 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

SSX (C-7): sc-166595 is recommended as a control antibody for monitoring of SSX1 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 SSX1 gene expression knockdown using RT-PCR Primer: SSX1 (h)-PR: sc-44120-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.