

SAP 145 siRNA (h): sc-38316

BACKGROUND

SF3b is a U2 snRNP-associated protein complex essential for spliceosome assembly. SF3b contains the spliceosomal proteins SAP 49, SAP 130, SAP 145, and SAP 155. SAP 130, SAP 145, and SAP 155 are present in a protein complex in HeLa nuclear extracts and associate with one another. While SAP 155 and SAP 130 interact with each other (directly or indirectly) within this complex, SAP 49 and SAP 145 are known to interact directly with each other. Unexpectedly, the SAP 49-SAP 145 protein-protein interaction requires the amino-terminus of SAP 49, which contains two RNA-recognition motifs. The observation that SAP 49 and SAP 145 interact directly with both U2 snRNP and the pre-mRNA suggests that this protein complex plays a role in tethering U2 snRNP to the branch site.

REFERENCES

1. Champion-Arnaud, P., et al. 1994. The prespliceosome components SAP 49 and SAP 145 interact in a complex implicated in tethering U2 snRNP to the branch site. *Genes Dev.* 8: 1974-1983.
2. Wells, S.E., et al. 1996. CUS1, a suppressor of cold-sensitive U2 snRNA mutations, is a novel yeast splicing factor homologous to human SAP 145. *Genes Dev.* 10: 220-232.
3. Igel, H., et al. 1998. Conservation of structure and subunit interactions in yeast homologues of splicing factor 3b (SF3b) subunits. *RNA* 4: 1-10.
4. Das, B.K., et al. 1999. Characterization of a protein complex containing spliceosomal proteins SAPs 49, 130, 145, and 155. *Mol. Cell. Biol.* 19: 6796-6802.
5. Kramer, A., et al. 1999. Combined biochemical and electron microscopic analyses reveal the architecture of the mammalian U2 snRNP. *J. Cell Biol.* 145: 1355-1368.

CHROMOSOMAL LOCATION

Genetic locus: SF3B2 (human) mapping to 11q13.1.

PRODUCT

SAP 145 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 SAP 145 shRNA Plasmid (h): sc-38316-SH and SAP 145 shRNA (h) Lentiviral Particles: sc-38316-V as alternate gene silencing products.

For independent verification of SAP 145 (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-38316A, sc-38316B and sc-38316C.

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.

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

SAP 145 siRNA (h) is recommended for the inhibition of SAP 145 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

SAP 145 (C-12): sc-514930 is recommended as a control antibody for monitoring of SAP 145 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 SAP 145 gene expression knockdown using RT-PCR Primer: SAP 145 (h)-PR: sc-38316-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.