

SAP 61 siRNA (m): sc-76444

BACKGROUND

SAP 61, also known as SF3A3 (splicing factor 3A subunit 3), PRP9, PRPF9 or SF3a60, is a 501 amino acid protein that contains one matrin-type zinc finger and belongs to the SF3A3 family. Localized to the nucleus, SAP 61 is a subunit of the SF3A splicing factor, a heterotrimeric complex comprised of three subunits that act in tandem to mediate the binding of U2 snRNP to the branchpoint sequence (BPS) in pre-mRNA. The SF3A complex is necessary for the conversion of 15S U2 snRNP into the active 17S protein that performs directly in pre-mRNA splicing events. Functioning as the third subunit of the complex, SAP 61 interacts with subunit 1 (SAP 114) via its N-terminus, while simultaneously binding to 15S U2 snRNP via its zinc finger domain. As is the case for all SF3A subunits, SAP 61 is essential for prespliceosome assembly and cell viability. In addition, a pseudogene exists for SAP 61 on chromosome 20.

REFERENCES

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2. Chiara, M.D., et al. 1994. Specific protein-protein interactions between the essential mammalian spliceosome-associated proteins SAP 61 and SAP 114. *Proc. Natl. Acad. Sci. USA* 91: 6403-6407.
3. Online Mendelian Inheritance in Man, OMIM[™]. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 605596. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Nesci, D., et al. 2004. A role for Cajal bodies in the final steps of U2 SnRNP biogenesis. *J. Cell Sci.* 117: 4423-4433.
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6. Tanackovic, G. and Krämer, A. 2005. Human splicing factor SF3A, but not SF1, is essential for pre-mRNA splicing *in vivo*. *Mol. Biol. Cell* 16: 1366-1377.
7. Dybkov, O., et al. 2006. U2 snRNA-protein contacts in purified human 17S U2 SnRNPs and in spliceosomal A and B complexes. *Mol. Cell. Biol.* 26: 2803-2816.

CHROMOSOMAL LOCATION

Genetic locus: Sf3a3 (mouse) mapping to 4 D2.2.

PRODUCT

SAP 61 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 SAP 61 shRNA Plasmid (m): sc-76444-SH and SAP 61 shRNA (m) Lentiviral Particles: sc-76444-V as alternate gene silencing products.

For independent verification of SAP 61 (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-76444A, sc-76444B and sc-76444C.

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 61 siRNA (m) is recommended for the inhibition of SAP 61 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

SAP 61 (H-3): sc-393673 is recommended as a control antibody for monitoring of SAP 61 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 61 gene expression knockdown using RT-PCR Primer: SAP 61 (m)-PR: sc-76444-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.