SANTA CRUZ BIOTECHNOLOGY, INC.

U2AF65 siRNA (m): sc-37668

BACKGROUND

SF3b is an U2 snRNP-associated protein complex essential for spliceosome assembly. SF3b contains the spliceosomal proteins SAPs 49, 130, 145 and 155. SAPs 130, 145 and 155 associate with one another to form a complex that is present in HeLa nuclear extracts. SAPs 49 and 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. SAP 49 and SAP 145 interact directly with both U2 snRNP and the pre-mRNA, which suggests that this protein complex plays a role in tethering U2 snRNP to the branch site. U2AF recruits SAP 49 to the branch point sequence during the initial steps of spliceosome assembly. U2AF exists as a heterodimer consisting of U2AF65 and U2AF35 and is required for splicing *in vivo*.

REFERENCES

1. Zamore, P.D., et al. 1989. Identification, purification and biochemical characterization of U2 small nuclear ribonucleoprotein auxiliary factor. Proc. Natl. Acad. Sci. USA 86: 9243-9247.
2. Kanaar, R., et al. 1993. The conserved pre-mRNA splicing factor U2AF from *Drosophila:* requirement for viability. Science 262: 569-573.
3. Potashkin, J., et al. 1993. U2AF homolog required for splicing *in vivo*. Science 262: 573-576.
4. 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.
5. 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.
6. Igel, H., et al. 1998. Conservation of structure and subunit interactions in yeast homologues of splicing factor 3b (SF3b) subunits. RNA 4: 1-10.
7. 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.

CHROMOSOMAL LOCATION

Genetic locus: U2af2 (mouse) mapping to 7 A1.

PRODUCT

U2AF65 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see U2AF65 shRNA Plasmid (m): sc-37668-SH and U2AF65 shRNA (m) Lentiviral Particles: sc-37668-V as alternate gene silencing products.

For independent verification of U2AF65 (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-37668A, sc-37668B and sc-37668C.

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

U2AF65 siRNA (m) is recommended for the inhibition of U2AF65 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 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

U2AF65 (MC3): sc-53942 is recommended as a control antibody for monitoring of U2AF65 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 U2AF65 gene expression knockdown using RT-PCR Primer: U2AF65 (m)-PR: sc-37668-PR (20 µl). 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.