SANTA CRUZ BIOTECHNOLOGY, INC.

PRP6 siRNA (h): sc-38207

BACKGROUND

Assembly of pre-mRNA spliceosomes requires the interaction between snRNPs U4/U6 and U5 to form the [U4/U6.U5] tri-snRNP. In yeast, the small nuclear ribonucleoprotein-associating protein, Prp6p is necessary for the accumulation of the [U4/U6.U5] tri-snRNP. Yeast Prp6p is uniquely located in discrete subnuclear regions, similar to the subnuclear localization of mammalian splicing components. Isolated from HeLa nuclear extract, mammalian PRP6 shares conserved tetrarico peptide repeats with yeast Prp6p, making PRP6 the mammalian homolog of yeast Prp6p. In contrast to yeast Prp6p, which is specific for U4/U6, the human PRP6 interacts within the tri-snRNP with both the U5 and the U4/U6 snRNPs via protein-protein interactions, thus providing a bridge that connects the two snRNP particles.

REFERENCES

1. Abovich, N., et al. 1990. The yeast PRP6 gene encodes a U4/U6 small nuclear ribonucleoprotein particle (snRNP), and the PRP9 gene encodes a protein required for U2 snRNP binding. Mol. Cell. Biol. 10: 6417-6425.
2. Blanton, S., et al. 1992. PRP38 encodes a yeast protein required for pre-mRNA splicing and maintenance of stable U6 small nuclear RNA levels. Mol. Cell. Biol. 12: 3939-3947.
3. Elliott, D.J., et al. 1992. A yeast splicing factor is localized in discrete subnuclear domains. EMBO J. 11: 3731-3736.
4. Galisson, F., et al. 1993. The biochemical defects of PRP4-1 and PRP6-1 yeast splicing mutants reveal that the PRP6 protein is required for the accumulation of the [U4/Ug.U5] tri-snRNP. Nucleic Acids Res. 21: 1555-1562.
5. Makarov, E.M., et al. 2000. The human homologue of the yeast splicing factor PRP6p contains multiple TPR elements and is stably associated with the U5 snRNP via protein-protein interactions. J. Mol. Biol. 298: 567-575.

CHROMOSOMAL LOCATION

Genetic locus: PRPF6 (human) mapping to 20q13.33.

PRODUCT

PRP6 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 PRP6 shRNA Plasmid (h): sc-38207-SH and PRP6 shRNA (h) Lentiviral Particles: sc-38207-V as alternate gene silencing products.

For independent verification of PRP6 (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-38207A, sc-38207B and sc-38207C.

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 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

PRP6 siRNA (h) is recommended for the inhibition of PRP6 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

PRP6 (B-1): sc-166889 is recommended as a control antibody for monitoring of PRP6 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 PRP6 gene expression knockdown using RT-PCR Primer: PRP6 (h)-PR: sc-38207-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.