SANTA CRUZ BIOTECHNOLOGY, INC.

PRP8 siRNA (m): sc-38210

BACKGROUND

PRP8, also designated pre-mRNA-processing-splicing factor 8, is a highly conserved nuclear protein and a central component of the catalytic core of the spliceosome, where it may be involved in various molecular rearrangements. PRP8, which is widely expressed, plays a role in transesterification reactions that regulate splicesome-induced pre-mRNA splicing. Specifically, PRP8 interacts with the GU dinucleotide at the 5' splice site (5'SS) and forms a specific UV-inducible cross-link. It also interacts functionally with the 3'SS, affecting the efficiency of the second catalytic step. PRP8 may play a role in the first transesterification step, as PRP8 mutations that prohibit negative regulation of PRP28 or PRP44/Brr2 subsequently block U4 activation. In addition, PRP8 interacts with a conserved region of U6 that is instrumental in the formation of the catalytic core of the spliceosome.

REFERENCES

1. Nagase, T., Ishikawa, K., Miyajima, N., Tanaka, A., Kotani, H., Nomura, N. and Ohara, O. 1998. Prediction of the coding sequences of unidentified human genes. IX. The complete sequences of 100 new cDNA clones from brain which can code for large proteins *in vitro*. DNA Res. 5: 31-39.
2. Collins, C.A. and Guthrie, C. 1999. Allele-specific genetic interactions between PRP8 and RNA active site residues suggest a function for Prp8 at the catalytic core of the spliceosome. Genes Dev. 13: 1970-1982.
3. Siatecka, M., Reyes, J.L. and Konarska, M.M. 1999. Functional interactions of PRP8 with both splice sites at the spliceosomal catalytic center. Genes Dev. 13: 1983-1993.
4. Vidal, V.P., Verdone, L., Mayes, A.E. and Beggs, J.D. 1999. Characterization of U6 snRNA-protein interactions. RNA 5: 1470-1481.
5. Grainger, R.J. and Beggs, J.D. 2005. PRP8 protein: at the heart of the spliceosome. RNA 11: 533-557.
6. Boon, K.L., Norman, C.M., Grainger, R.J., Newman, A.J. and Beggs, J.D. 2006. PRP8p dissection reveals domain structure and protein interaction sites. RNA 12: 198-205.

CHROMOSOMAL LOCATION

Genetic locus: Prpf8 (mouse) mapping to 11 B5.

PRODUCT

PRP8 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 PRP8 shRNA Plasmid (m): sc-38210-SH and PRP8 shRNA (m) Lentiviral Particles: sc-38210-V as alternate gene silencing products.

For independent verification of PRP8 (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-38210A, sc-38210B and sc-38210C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

PRP8 siRNA m) is recommended for the inhibition of PRP8 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

PRP8 (E-5): sc-55533 is recommended as a control antibody for monitoring of PRP8 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 PRP8 gene expression knockdown using RT-PCR Primer: PRP8 (m)-PR: sc-38210-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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