SANTA CRUZ BIOTECHNOLOGY, INC.

RBM8 siRNA (m): sc-38346

BACKGROUND

The exon junction complex (EJC) is a multiprotein complex that assembles approximately 20-24 nucleotides upstream of exon-exon junctions in pre-mRNAs. It is involved in mRNA export, cytoplasmic localization, and nonsense-mediated mRNA decay. Members of the EJC include Y14, Aly/REF, Magoh, RNPS1, SRm160, and DEK. Aly/REF, Magoh, and Y14, identified as RBM8 in mouse and rat, make up the core of the EJC, and these proteins remain stably bound to spliced mRNAs in the cytoplasm until they are translated. Therefore, Y14, Aly/REF, and Magoh have the ability to communicate to the cytoplasm the processing history of the mRNA, including the position of the removed introns. The gene encoding human Y14 encodes three transcripts. Y14 is a ubiquitously expressed protein. Although Y14 shuttles to the cytoplasm, it is predominantly detected in the nucleus and is colocalized with oskar mRNA at the posterior pole of the cell.

REFERENCES

1. Conklin, D.C., et al. 2000. Cloning and gene expression of a novel human ribonucleoprotein. Biochim. Biophys. Acta 1492: 465-469.
2. Zhao, X.F., et al. 2000. Magoh interacts with a novel RNA-binding protein. Genomics 63: 145-148.
3. Hachet, O. and Ephrussi, A. 2001. *Drosophila* Y14 shuttles to the posterior of the oocyte and is required for oskar mRNA transport. Curr. Biol. 11: 1666-1674.
4. Kataoka, N., et al. 2001. Magoh, a human homolog of *Drosophila* mago nashi protein, is a component of the splicing-dependent exon-exon junction complex. EMBO J. 20: 6424-6433.
5. Le Hir, H., et al. 2001. The protein Mago provides a link between splicing and mRNA localization. EMBO Rep. 2: 1119-1124.
6. Kim, V.N. and Dreyfus, G. 2001. Nuclear mRNA binding proteins couple pre-mRNA splicing and post-splicing events. Mol. Cells 12: 1-10.
7. Reichert, V.L., et al. 2002. 5' exon interactions within the human spliceosome establish a framework for exon junction complex structure and assembly. Genes Dev. 16: 2778-2791.
8. Dostie, J. and Dreyfuss, G. 2002. Translation is required to remove Y14 from mRNAs in the cytoplasm. Curr. Biol. 12: 1060-1067.

CHROMOSOMAL LOCATION

Genetic locus: Rbm8a (mouse) mapping to 3 F2.1.

PRODUCT

RBM8 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 RBM8 shRNA Plasmid (m): sc-38346-SH and RBM8 shRNA (m) Lentiviral Particles: sc-38346-V as alternate gene silencing products.

For independent verification of RBM8 (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-38346A, sc-38346B and sc-38346C.

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

RBM8 siRNA (m) is recommended for the inhibition of RBM8 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

Y14 (4C4): sc-32312 is recommended as a control antibody for monitoring of RBM8 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 RBM8 gene expression knockdown using RT-PCR Primer: RBM8 (m)-PR: sc-38346-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.