SANTA CRUZ BIOTECHNOLOGY, INC.

RBM9 siRNA (h): sc-76371

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The Power to Question

BACKGROUND

RBM9 (RNA binding motif protein 9), also known as RTA, fxh, FOX2, Fox-2, HNRBP2 or HRNBP2, is a 390 amino acid protein that contains one RRM (RNA recognition motif) domain. RBM9 is thought to be a key regulator of alternative exon splicing in the nervous system and other cell types. RBM9 regulates the splicing activity of the highly conserved RNA 5'-UGCAUGU-3' element, an intron splicing enhancer that is often located adjacent to tissue-specific alternative exons. RBM9 prevents binding of U2AF65 (U2 snRNP auxiliary factor large subunit) to the 3' splice site of the RNA splicing element which affects alternative splicing of tissue-specific exons. RBM9 also interacts with the ERα (estrogen receptor α) transcription factor and regulates ERα transcriptional activity. Eight isoforms of RBM9 exists due to alternative splicing events.

REFERENCES

1. Underwood, J.G., et al. 2005. Homologues of the *Caenorhabditis elegans* Fox-1 protein are neuronal splicing regulators in mammals. Mol. Cell. Biol. 25: 10005-10016.
2. Minovitsky, S., et al. 2005. The splicing regulatory element, UGCAUG, is phylogenetically and spatially conserved in introns that flank tissue-specific alternative exons. Nucleic Acids Res. 33: 714-724.
3. Ponthier, J.L., et al. 2006. Fox-2 splicing factor binds to a conserved intron motif to promote inclusion of protein 4.1R alternative exon 16. J. Biol. Chem. 281: 12468-12474.
4. Zhou, H.L., et al. 2007. Role for Fox-1/Fox-2 in mediating the neuronal pathway of calcitonin/calcitonin gene-related peptide alternative RNA processing. Mol. Cell. Biol. 2: 830-841.
5. Yang, G., et al. 2008. Regulated Fox-2 isoform expression mediates protein 4.1R splicing during erythroid differentiation. Blood 111: 392-401.
6. Zhang, C., et al. 2008. Defining the regulatory network of the tissue-specific splicing factors Fox-1 and Fox-2. Genes Dev. 22: 2550-2563.
7. Zhou, H.L. and Lou, H. 2008. Repression of prespliceosome complex formation at two distinct steps by Fox-1/Fox-2 proteins. Mol. Cell. Biol. 28: 5507-5516.

CHROMOSOMAL LOCATION

Genetic locus: RBFOX2 (human) mapping to 22q12.3.

PRODUCT

RBM9 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 RBM9 shRNA Plasmid (h): sc-76371-SH and RBM9 shRNA (h) Lentiviral Particles: sc-76371-V as alternate gene silencing products.

For independent verification of RBM9 (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-76371A, sc-76371B and sc-76371C.

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

RBM9 siRNA (h) is recommended for the inhibition of RBM9 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

RBM9 (F-8): sc-271407 is recommended as a control antibody for monitoring of RBM9 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 RBM9 gene expression knockdown using RT-PCR Primer: RBM9 (h)-PR: sc-76371-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.