

## 9G8 siRNA (m): sc-38247

### BACKGROUND

Pre-mRNA splicing enhancer elements are short RNA sequences capable of activating weak splice sites in nearby introns, and they are required for accurate splice site recognition and the control of alternative splicing. Splicing enhancer elements contain specific binding sites for serine/arginine (SR)-rich splicing factors, which include SC35, 9G8, SRp20, and SF2/ASF. The family of SR factors all contain one or more RNA recognition motifs (RRM) and an arginine/serine (RS)-rich domain, and they are essential for constitutive splicing and also regulate splicing in a concentration-dependent manner by influencing the selection of alternative splice sites. The majority of SR proteins, including SC35 and SRp40, are confined to the nucleus, while SF2/ASF, SRp20, and 9G8 are continuously shuttled between the nucleus and the cytoplasm and contribute to mRNA transport. The activity of SR proteins in regulated splicing is antagonized by members of the hnRNP A/B family of proteins, which induce drastic shifts in the selection of splicing-sites. An additional SR-associated protein, p32, tightly associates with SR factors and preferentially inhibits ASF/SF2 functioning as both a splicing enhancer and splicing repressor protein by preventing the stable interaction of ASF/SF2 and the RNA.

### REFERENCES

1. Fu, X.D. 1993. Specific commitment of different pre-mRNAs to splicing by single SR proteins. *Nature* 365: 82-85.
2. Mayeda, A., et al. 1994. Function of conserved domains of hnRNP A1 and other hnRNP A/B proteins. *EMBO J.* 13: 5483-5495.
3. Jumaa, H., et al. 1997. The splicing factor SRp20 modifies splicing of its own mRNA and ASF/SF2 antagonizes this regulation. *EMBO J.* 16: 5077-5085.
4. Caceres, J.F., et al. 1998. A specific subset of SR proteins shuttles continuously between the nucleus and the cytoplasm. *Genes Dev.* 12: 55-66.
5. Schaal, T.D., et al. 1999. Selection and characterization of pre-mRNA splicing enhancers: identification of novel SR protein-specific enhancer sequences. *Mol. Cell. Biol.* 19: 1705-1719.
6. Petersen-Mahrt, S.K., et al. 1999. The splicing factor-associated protein, p32, regulates RNA splicing by inhibiting ASF/SF2 RNA binding and phosphorylation. *EMBO J.* 18: 1014-1024.

### CHROMOSOMAL LOCATION

Genetic locus: Sfrs7 (mouse) mapping to 17 E3.

### PRODUCT

9G8 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see 9G8 shRNA Plasmid (m): sc-38247-SH and 9G8 shRNA (m) Lentiviral Particles: sc-38247-V as alternate gene silencing products.

For independent verification of 9G8 (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-38247A, sc-38247B and sc-38247C.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

9G8 siRNA (m) is recommended for the inhibition of 9G8 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  $\mu$ M in 66  $\mu$ l. 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

9G8/SRp20 (G-3): sc-390126 is recommended as a control antibody for monitoring of 9G8 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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor 9G8 gene expression knockdown using RT-PCR Primer: 9G8 (m)-PR: sc-38247-PR (20  $\mu$ l, 425 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### SELECT PRODUCT CITATIONS

1. Gu, J., et al. 2012. Cyclic AMP-dependent protein kinase regulates 9G8-mediated alternative splicing of Tau exon 10. *FEBS Lett.* 586: 2239-2244.

### RESEARCH USE

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