SANTA CRUZ BIOTECHNOLOGY, INC.

MBNL1 siRNA (m): sc-60989

BACKGROUND

Pre-mRNA splicing is a critical step in the posttranscriptional regulation of gene expression. Several protein complexes are involved in proper mRNA splicing and transport. The muscleblind proteins, MBNL1, MBNL2 and MBNL3, promote inclusion or exclusion of specific exons on different pre-mRNAs by antagonizing the activity of CUG-BP and ETR3-like factors bound to distinct intronic sites. MBNL1 is a deduced 370 amino acid protein which is predominantly expressed in skeletal muscle, prostate, lung, heart, small intestine, ovary and placenta tissues. MBNL1 and MBNL2, which associate with expanded CUG repeats *in vitro,* both localize to the nuclear foci in both DM1 and DM2 (myotonic dystrophy types 1 and 2), suggesting that the nuclear accumulation of mutant RNA is pathogenic in DM1, therefore implicating MBNL1 and 2 in the pathogenesis of both disorders.

REFERENCES

1. Ishikawa, K., et al. 1998. Prediction of the coding sequences of unidentified human genes. VIII. 78 new cDNA clones from brain which code for large proteins *in vitro.* DNA Res. 4: 307-313.
2. Miller, J.W., et al. 2000. Recruitment of human muscleblind proteins to (CUG)(n) expansions associated with myotonic dystrophy. EMBO J. 19: 4439-4448.
3. Mankodi, A., et al. 2001. Muscleblind localizes to nuclear foci of aberrant RNA in myotonic dystrophy types 1 and 2. Hum. Mol. Genet. 10: 2165-2170.
4. Fardaei, M., et al. 2002. Three proteins, MBNL, MBLL and MBXL, co-localize *in vivo* with nuclear foci of expanded-repeat transcripts in DM1 and DM2 cells. Hum. Mol. Genet. 11: 805-814.
5. Ho, T.H., et al. 2005. Co-localization of muscleblind with RNA foci is separable from mis-regulation of alternative splicing in myotonic dystrophy. J. Cell Sci. 118: 2923-2933.
6. Ladd, A.N., et al. 2005. Dynamic balance between activation and repression regulates pre-mRNA alternative splicing during heart development. Dev. Dyn. 233: 783-793.

CHROMOSOMAL LOCATION

Genetic locus: Mbnl1 (mouse) mapping to 3 D.

PRODUCT

MBNL1 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 MBNL1 shRNA Plasmid (m): sc-60989-SH and MBNL1 shRNA (m) Lentiviral Particles: sc-60989-V as alternate gene silencing products.

For independent verification of MBNL1 (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-60989A, sc-60989B and sc-60989C.

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

MBNL1 siRNA (m) is recommended for the inhibition of MBNL1 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

MBNL1 (3A4): sc-47740 is recommended as a control antibody for monitoring of MBNL1 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 MBNL1 gene expression knockdown using RT-PCR Primer: MBNL1 (m)-PR: sc-60989-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.