

# MBNL2 siRNA (m): sc-60991

## BACKGROUND

Pre-mRNA splicing is a critical step in the post-transcriptional 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 ETR-3-like factors bound to distinct intronic sites. MBNL1 and MBNL2, associate with expanded CUG repeats *in vitro* and 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 muscleblind proteins MBNL1 and MBNL2 in the pathogenesis of both disorders. MBNL2, a 367 amino acid protein, participates in recruitment of Intergrin  $\alpha 3$  to focal adhesions in a RNA-dependent protein localization mechanism.

## 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. Colocalization of muscleblind with RNA foci is separable from mis-regulation of alternative splicing in myotonic dystrophy. J. Cell Sci. 118: 2923-2933.
6. Ladd, AN. 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: Mbnl2 (mouse) mapping to 14 E4.

## PRODUCT

MBNL2 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 MBNL2 shRNA Plasmid (m): sc-60991-SH and MBNL2 shRNA (m) Lentiviral Particles: sc-60991-V as alternate gene silencing products.

For independent verification of MBNL2 (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-60991A, sc-60991B and sc-60991C.

## 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

MBNL2 siRNA (m) is recommended for the inhibition of MBNL2 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

MBNL2 (3B4): sc-136167 is recommended as a control antibody for monitoring of MBNL2 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 MBNL2 gene expression knockdown using RT-PCR Primer: MBNL2 (m)-PR: sc-60991-PR (20  $\mu$ l). 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](http://www.scbt.com) for detailed protocols and support products.