

p54/nrb siRNA (m): sc-38164

BACKGROUND

Found in both primary and transformed human cells, paraspeckles are discrete bodies in the interchromatin nucleoplasmic space which contain p54/nrb (nuclear RNA-binding protein) and at least two other RNA binding proteins, paraspeckle protein 1 (PSP1) and paraspeckle protein 2 (PSP2). Paraspeckles often co-localize with splicing speckles, which are the site of splicing factor accumulation. Paraspeckle proteins, including p54/nrb, move dynamically between the nucleolus and paraspeckles and translocate to distinct caps in the nucleolar periphery when transcription is inhibited. Originally purified from HeLa cells, the nuclear p54/nrb has two RNA recognition motifs and shares extensive homology with both the human splicing factor PSF and *Drosophila* NONA/BJ6, which is required for normal vision and courtship. The shared domain between these proteins is termed a DBHS (*Drosophila* behavior, human splicing) domain and may play a role in regulating various pathways at the level of pre-mRNA splicing.

REFERENCES

1. Dong, B., et al. 1993. Purification and cDNA cloning of HeLa cell p54^{nrb}, a nuclear protein with two RNA recognition motifs and extensive homology to human splicing factor PSF and *Drosophila* NONA/BJ6. *Nucleic Acids Res.* 21: 4085-4092.
2. Brown, C.J., et al. 1997. Expression of genes from the human active and inactive X chromosomes. *Am. J. Hum. Genet.* 60: 1333-1343.
3. Zhang, Z. and Carmichael, G.G. 2001. The fate of dsRNA in the nucleus: a p54^{nrb}-containing complex mediates the nuclear retention of promiscuously A-to-I edited RNAs. *Cell* 106: 465-475.

CHROMOSOMAL LOCATION

Genetic locus: Nono (mouse) mapping to X D.

PRODUCT

p54/nrb 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p54/nrb shRNA Plasmid (m): sc-38164-SH and p54/nrb shRNA (m) Lentiviral Particles: sc-38164-V as alternate gene silencing products.

For independent verification of p54/nrb (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-38164A, sc-38164B and sc-38164C.

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

APPLICATIONS

p54/nrb siRNA (m) is recommended for the inhibition of p54/nrb 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 μ M in 66 μ 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

p54/nrb (F-5): sc-376804 is recommended as a control antibody for monitoring of p54/nrb 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 p54/nrb gene expression knockdown using RT-PCR Primer: p54/nrb (m)-PR: sc-38164-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kleene, R., et al. 2023. The KDET motif in the intracellular domain of the cell adhesion molecule L1 interacts with several nuclear, cytoplasmic, and mitochondrial proteins essential for neuronal functions. *Int. J. Mol. Sci.* 24: 932.

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.