SANTA CRUZ BIOTECHNOLOGY, INC.

hnRNP D0 siRNA (m): sc-37029

BACKGROUND

Heterogeneous nuclear ribonucleoproteins (hnRNPs) constitute a set of poly-peptides that contribute to mRNA transcription, pre-mRNA processing, mature mRNA transport to the cytoplasm and translation. They also bind heterogeneous nuclear RNA (hnRNA), which are the transcripts produced by RNA polymerase II. There are approximately 20 known hnRNP proteins, and their complexes are the major constituents of the spliceosome. The majority of hnRNP proteins components are localized to the nucleus; however some shuttle between the nucleus and the cytoplasm. Two abundant and ubiquitously expressed members of the hnRNP family are hnRNP D0 and hnRNP R. Specifically, the hnRNP D0 protein contains two RNA-binding domains (RBDs), which bind to both RNA and DNA sequences. hnRNP D0 also posesses a transactivator domain and is involved in transcriptional regulation.

REFERENCES

1. Badolato, J., et al. 1995. Identification and characterisation of a novel human RNA-binding protein. Gene 166: 323-327.
2. Siomi, H. and Dreyfuss, G. 1995. A nuclear localization domain in the hnRNP A1 protein. J. Cell Biol. 129: 551-560.
3. Kajita, Y., et al. 1995. The UUAG-specific RNA binding protein, heterogeneous nuclear ribonucleoprotein D0. Common modular structure and binding properties of the 2xRBD-Gly family. J. Biol. Chem. 270: 22167-22175.
4. Hassfeld, W., et al. 1998. Molecular definition of heterogeneous nuclear ribonucleoprotein R (hnRNP R) using autoimmune antibody: immunological relationship with hnRNP P. Nucleic Acids Res. 26: 439-445.
5. Nagata, T., et al. 1999. Structure and interactions with RNA of the N-terminal UUAG-specific RNA-binding domain of hnRNP D0. J. Mol. Biol. 287: 221-237.
6. Kim, J.H., et al. 2000. Protein-protein interaction among hnRNPs shuttling between nucleus and cytoplasm. J. Mol. Biol. 298: 395-405.
7. Melcak, I., et al. 2000. Nuclear pre-mRNA compartmentalization: trafficking of released transcripts to splicing factor reservoirs. Mol. Biol. Cell 11: 497-510.
8. Tolnay, M., et al. 2000. Heterogeneous nuclear ribonucleoprotein D0 contains transactivator and DNA-binding domains. Biochem. J. 1: 151-158.

CHROMOSOMAL LOCATION

Genetic locus: Hnrnpd (mouse) mapping to 5 E4.

PRODUCT

hnRNP D0 siRNA (m) is a pool of 2 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 hnRNP D0 shRNA Plasmid (m): sc-37029-SH and hnRNP D0 shRNA (m) Lentiviral Particles: sc-37029-V as alternate gene silencing products.

For independent verification of hnRNP D0 (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-37029A and sc-37029B.

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

hnRNP D0 siRNA (m) is recommended for the inhibition of hnRNP D0 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

pan hnRNP (C-6): sc-166577 is recommended as a control antibody for monitoring of hnRNP D0 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 hnRNP D0 gene expression knockdown using RT-PCR Primer: hnRNP D0 (m)-PR: sc-37029-PR (20 µl, 461 bp). 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.