SANTA CRUZ BIOTECHNOLOGY, INC.

Rent2 siRNA (m): sc-38226

BACKGROUND

In eukaryotes, it is essential to have the ability to detect and degrade transcripts that lack full coding potential. Nonsense-mediated RNA decay (NMD) protects the organism by avoiding the translation of truncated peptides with dominant negative or deleterious gain-of-function potential. Rent1, a mammalian ortholog of Upflp, is essential for embryonic viability. Rent1 (also designated regulator of nonsense transcripts and HUpf1) contains an N-terminal zinc finger-like domain, NTPase domains and a region comprised of domains that define Rent1 as a superfamily group I helicase. Rent1 protein has nucleic-acid-dependent ATPase activity and 5' to 3' helicase activity. In addition, Rent1 is an RNA-binding protein whose activity is modulated by ATP and directly interacts with Rent2, which is a mammalian homolog of Upf2p. Two mammalian orthologs to Upf3p, Rent3a and Rent3b, are encoded by two separate genes. Rent3b (also known as Rent3X) is encoded by a X-linked gene and localizes primarily to the nucleus, while Rent1 and Rent2 localize primarily in the cytoplasm. Specific Rent3 protein interactions with Y14 and spliced mRNA suggest Rent3a and Rent3b serve as a link between splicing and NMD in the cytoplasm.

REFERENCES

1. Perlick, H.A., et al. 1996. Mammalian orthologues of a yeast regulator of nonsense transcript stability. Proc. Natl. Acad. Sci. USA 93: 10928-10932.
2. Page, M.F., et al. 1999. SMG-2 is a phosphorylated protein required for mRNA surveillance in *Caenorhabditis elegans* and related to Upf1p of yeast. Mol. Cell. Biol. 19: 5943-5951.
3. Bhattacharya, A., et al. 2000. Characterization of the biochemical properties of the human Upf1 gene product that is involved in nonsense-mediated mRNA decay. RNA 6: 1226-1235.
4. Mendell, J.T., et al. 2000. Novel Upf2p orthologues suggest a functional link between translation initiation and nonsense surveillance complexes. Mol. Cell. Biol. 20: 8944-8957.
5. Serin, G., et al. 2001. Identification and characterization of human orhtologues to *Saccharomyces cerevisiae* Upf2 protein and Upf3 protein *(Caenorhabditis elegans* SMG-4). Mol. Cell. Biol. 21: 209-223.

CHROMOSOMAL LOCATION

Genetic locus: Upf2 (mouse) mapping to 2 A1.

PRODUCT

Rent2 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 Rent2 shRNA Plasmid (m): sc-38226-SH and Rent2 shRNA (m) Lentiviral Particles: sc-38226-V as alternate gene silencing products.

For independent verification of Rent2 (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-38226A, sc-38226B and sc-38226C.

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

Rent2 siRNA (m) is recommended for the inhibition of Rent2 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

Rent2 (G-10): sc-374230 is recommended as a control antibody for monitoring of Rent2 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 Rent2 gene expression knockdown using RT-PCR Primer: Rent2 (m)-PR: sc-38226-PR (20 µl). 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.