SANTA CRUZ BIOTECHNOLOGY, INC.

Dicer siRNA (r): sc-270275

BACKGROUND

The mammalian Dicer is a type III RNase-related protein with orthologs in yeast, *Drosophila* and *Arabidopsis*. Dicer contains an RNA-helicase motif including a DEXH-box in its amino-terminus and an RNase motif in the carboxy-terminus. The gene encoding human Dicer maps to chromosome 14q31. Dicer is expressed in brain, heart, liver, lung, pancreas, kidney and placenta and functions in the RNA interference pathway. Dicer cleaves short hairpin RNA precursors of approximately 70 bp into 21-23 bp dsRNAs that selectively target the destruction of homologous RNAs. Dicer localizes to the cytoplasm of mammalian cells. Specifically, it co-localizes with calreticulin in the endoplasmic reticulum. Although the cleavage of RNA by Dicer is ATP-independent, the product release necessary for the rapid turnover of this enzyme may be attributed to ATP. Immunoprecipitation studies indicate Dicer forms a complex with PIWI domain of eIF2C translation initiation factors.

REFERENCES

1. Matsuda, S., et al. 2000. Molecular cloning and characterization of a novel human gene (HERNA) which encodes a putative RNA-helicase. Biochim. Biophys. Acta 1490: 163-169.
2. Billy, E., et al. 2001. Specific interference with gene expression induced by long, double-stranded RNA in mouse embryonal teratocarcinoma cell lines. Proc. Natl. Acad. Sci. USA 98: 14428-14433.
3. Provost, P., et al. 2002. Ribonuclease activity and RNA binding of recombinant human Dicer. EMBO J. 21: 5864-5874.
4. Paddison, P.J., et al. 2002. Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. Genes Dev. 16: 948-958.
5. Zhang, H., et al. 2002. Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP. EMBO J. 21: 5875-5885.
6. Doi, N., et al. 2004. Short-interfering-RNA-mediated gene silencing in mammalian cells requires Dicer and eIF2C translation initiation factors. Curr. Biol. 13: 41-46.

CHROMOSOMAL LOCATION

Genetic locus: Dicer1 (rat) mapping to 6q32.

PRODUCT

Dicer siRNA (r) 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 Dicer shRNA Plasmid (r): sc-270275-SH and Dicer shRNA (r) Lentiviral Particles: sc-270275-V as alternate gene silencing products.

For independent verification of Dicer (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270275A, sc-270275B and sc-270275C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

Dicer siRNA (r) is recommended for the inhibition of Dicer expression in rat 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

Dicer (F-10): sc-136979 is recommended as a control antibody for monitoring of Dicer 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 Dicer gene expression knockdown using RT-PCR Primer: Dicer (r)-PR: sc-270275-PR (20 µl, 581 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.