

eIF2C4 siRNA (m): sc-44668

BACKGROUND

Eukaryotic translation initiation factor 2C (eIF2C) proteins (argonaute family) influence RNA interference (RNAi) as components of the RNA-inducible silencing complex (RISC) or microRNA (miRNA)-containing ribonucleoprotein particle (miRNP). Small RNAs, including small interfering RNAs (siRNAs) and miRNAs, can silence target genes through mechanisms that utilize RISC or miRNP particles. eIF2C1 (argonaute 1, AGO1, eIF2C, GERP95, Q99) and Dicer1 play a coordinated role in siRNA-mediated gene silencing. eIF2C2 (Slicer, argonaute 2, AGO2, Q10) is a RISC component that can concentrate in cytoplasmic processing bodies (P-bodies) and catalyze mRNA cleavage. Mammalian P-bodies contain mRNAs and have an association with miRNA-induced translational silencing and siRNA-induced mRNA degradation. Additional eIF2C proteins include eIF2C3 (argonaute 3, AGO3), eIF2C4 (argonaute 4, AGO4) and mEIF2c5 (mouse argonaute 5).

REFERENCES

1. Martinez, J., et al. 2002. Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. *Cell* 110: 563-574.
2. Carmell, M.A., et al. 2002. The Argonaute family: tentacles that reach into RNAi, developmental control, stem cell maintenance and tumorigenesis. *Genes Dev.* 16: 2733-2742.
3. Yan, K.S., et al. 2003. Structure and conserved RNA binding of the PAZ domain. *Nature* 426: 468-474.
4. Meister, G., et al. 2004. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol. Cell* 15: 185-197.
5. Sontheimer, E.J., et al. 2004. Molecular biology. Argonaute journeys into the heart of RISC. *Science* 305: 1409-1410.
6. Liu, J., et al. 2004. Argonaute 2 is the catalytic engine of mammalian RNAi. *Science* 305: 1437-1441.
7. Sen, G.L., et al. 2005. Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nat. Cell Biol.* 7: 633-636.
8. Liu, J., et al. 2005. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat. Cell Biol.* 7: 719-723.

CHROMOSOMAL LOCATION

Genetic locus: Ago4 (mouse) mapping to 4 D2.2.

PRODUCT

eIF2C4 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 eIF2C4 shRNA Plasmid (m): sc-44668-SH and eIF2C4 shRNA (m) Lentiviral Particles: sc-44668-V as alternate gene silencing products.

For independent verification of eIF2C4 (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-44668A, sc-44668B and sc-44668C.

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

eIF2C4 siRNA (m) is recommended for the inhibition of eIF2C4 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

eIF2C (B-3): sc-376696 is recommended as a control antibody for monitoring of eIF2C4 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 eIF2C4 gene expression knockdown using RT-PCR Primer: eIF2C4 (m)-PR: sc-44668-PR (20 μ 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 for detailed protocols and support products.