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eIF2C3 shRNA (m) Lentiviral Particles: sc-44660-V

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 melF2c5 (mouse argonaute 5).

REFERENCES

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- 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.
- Yan, K.S., et al. 2003. Structure and conserved RNA binding of the PAZ domain. *Nature* 426: 468-474.
- Meister, G., et al. 2004. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol. Cell* 15: 185-197.
- Sontheimer, E.J., et al. 2004. Molecular biology. Argonaute journeys into the heart of RISC. *Science* 305: 1409-1410.
- Liu, J., et al. 2004. Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 305: 1437-1441.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: Eif2c3 (mouse) mapping to 4 D2.2.

PRODUCT

eIF2C3 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see eIF2C3 siRNA (m): sc-44660 and eIF2C3 shRNA Plasmid (m): sc-44660-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

eIF2C3 shRNA (m) Lentiviral Particles is recommended for the inhibition of eIF2C3 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

eIF2C3 (N-12): sc-32660 is recommended as a control antibody for monitoring of eIF2C3 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor eIF2C3 gene expression knockdown using RT-PCR Primer: eIF2C3 (m)-PR: sc-44660-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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