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eIF4AIII shRNA (h) Lentiviral Particles: sc-44528-V

BACKGROUND

The eukaryotic translation factor 4A (eIF4A) is a member of DEA(D/H)-box RNA helicase family that couples ATP hydrolysis to RNA binding and duplex separation. eIF4A participates in the initiation of translation by unwinding secondary structure in the 5'-untranslated region of mRNAs and facilitating scanning by the 40 S ribosomal subunit for the initiation codon. eIF4AIII is a component of the exon junction complex (EJC) that assembles near exon-exon junctions of mRNAs as a result of splicing. eIF4AIII, but not eIF4AI or eIF4AII, preferentially associates with spliced mRNA. eIF4AIII is found in the nucleus whereas eIF4AI and eIF4AII are found in the cytoplasm.

REFERENCES

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3. Rogers, G.W., Jr., et al. 2001. Further characterization of the helicase activity of eIF4A. Substrate specificity. *J. Biol. Chem.* 276: 12598-12608.
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CHROMOSOMAL LOCATION

Genetic locus: EIF4A3 (human) mapping to 17q25.3.

PRODUCT

eIF4AIII shRNA (h) 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 eIF4AIII siRNA (h): sc-44528 and eIF4AIII shRNA Plasmid (h): sc-44528-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

eIF4AIII shRNA (h) Lentiviral Particles is recommended for the inhibition of eIF4AIII expression in human 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

eIF4AIII (B-2): sc-365549 is recommended as a control antibody for monitoring of eIF4AIII 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor eIF4AIII gene expression knockdown using RT-PCR Primer: eIF4AIII (h)-PR: sc-44528-PR (20 μ l, 506 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.

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