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eIF5A2 siRNA (m): sc-144623

BACKGROUND

In mammalian cells, translation is controlled at the level of polypeptide chain initiation by eukaryotic initiation factors. The human eukaryotic translation initiation factor 5A gene, also designated eIF-4D or eIF5A1, maps to chromosome 17p131 and encodes a 154 amino acid protein that is linked to cellular polyamine homeostasis. eIF5A1 localizes to the nuclear and cytoplasmic compartments of mammalian cells where it can stimulate ribosomal peptidyl-transferase and may be involved in nucleocytoplasmic mRNA transport and/or protein translation. eIF5A1 contains a unique spermidine-derived post-translational modification at Lys-50, hypusine, which is necessary for eIF5A1's biochemical activity and for cellular proliferative signaling. In addition, eIF5A1 is a cellular cofactor for the function of the Rev transactivator protein of human immunodeficiency virus type 1 (HIV-1). Inhibition of eIF5A1 interaction with Rev leads to a block of the viral replication cycle. A highly-conserved protein that is found in all living organisms, eIF5A2 (eukaryotic translation initiation factor 5A-2) is a 153 amino acid protein that has 94% sequence similarity to eIF5A1 and also shares the hypusine residue. Amplification of the gene encoding eIF5A2 is observed in ovarian carcinomas and overexpression of eIF5A2 is linked to advanced stages of ovarian cancers.

REFERENCES

1. Smit-McBride, Z., et al. 1989. Sequence determination and cDNA cloning of eukaryotic initiation factor 4D, the hypusine-containing protein. *J. Biol. Chem.* 264: 1578-1583.
2. Hershey, J.W. 1991. Translational control in mammalian cells. *Ann. Rev. Biochem.* 60: 717-755.
3. Merrick, W.C. 1992. Mechanism and regulation of eukaryotic protein synthesis. *Microbiol. Rev.* 56: 291-315.
4. Bevec, D., et al. 1994. Induced gene expression of the hypusine-containing protein eukaryotic initiation factor 5A in activated human T lymphocytes. *Proc. Natl. Acad. Sci. USA* 91: 10829-10833.
5. Steinkasserer, A., et al. 1995. The eukaryotic cofactor for the human immunodeficiency virus type 1 (HIV-1) rev protein, eIF-5A, maps to chromosome 17p12-p13: three eIF-5A pseudogenes map to 10q23.3, 17q25, and 19q13.2. *Genomics* 25: 749-752.

CHROMOSOMAL LOCATION

Genetic locus: Eif5a2 (mouse) mapping to 3 A3.

PRODUCT

eIF5A2 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 eIF5A2 shRNA Plasmid (m): sc-144623-SH and eIF5A2 shRNA (m) Lentiviral Particles: sc-144623-V as alternate gene silencing products.

For independent verification of eIF5A2 (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-144623A, sc-144623B and sc-144623C.

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

eIF5A2 siRNA (m) is recommended for the inhibition of eIF5A2 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

eIF5A (H-8): sc-390202 is recommended as a control antibody for monitoring of eIF5A2 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 eIF5A2 gene expression knockdown using RT-PCR Primer: eIF5A2 (m)-PR: sc-144623-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.