SANTA CRUZ BIOTECHNOLOGY, INC.

eIF5 siRNA (h): sc-35288

BACKGROUND

In mammalian cells, translation is controlled at the level of polypeptide chain initiation by initiation factors. The eukaryotic translation initiation factor 5 (eIF5) catalyzes the hydrolysis of GTP bound to the 40S ribosomal subunit, a function necessary for the subsequent joining of the 40S and 60S subunits to form the 80S initiation complex. eIF-4E specifically binds to the mRNA cap to promote unwinding and exposure of the AUG-initiation codon. Overexpression of eIF-4E can lead to cell transformation and tumorigenesis. An additional initiation factor, eIF-2, is present as a heterotrimer composed of eIF-2α, eIF-2β and eIF-2γ subunits. This heterotrimer forms a complex with GTP and tRNA which then binds to the 40S ribosomal subunit. After the formation of the 80S initiation complex, eIF-2 is hydrolyzed and eIF-2-GDP is released from the complex. eIF-2-GDP is subsequently converted to eIF-2-GTP, a reaction catalyzed by eIF-2B, and is then available to catalyze another round of initiation.

REFERENCES

1. Kozak, M. 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes and organelles. Microbiol. Rev. 47: 1-45.
2. Ernst, H., et al. 1987. Cloning and sequencing of complementary DNAs encoding the α-subunit of translational initiation factor eIF2. Characterization of the protein and its messenger RNA. J. Biol. Chem. 262: 1206-1212.
3. Hershey, J.W. 1991. Translational control in mammalian cells. Annu. Rev. Biochem. 60: 717-755.
4. Merrick, W.C. 1992. Mechanism and regulation of eukaryotic protein synthesis. Microbiol. Rev. 56: 291-315.
5. Rinker-Schaeffer, C.W., et al. 1993. Decreasing the level of translation initiation factor 4E with antisense RNA causes reversal of Ras-mediated transformation and tumorigenesis of cloned rat embryo fibroblasts. Int. J. Cancer 55: 841-847.
6. Pause, A., et al. 1994. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. Nature 371: 762-767.

CHROMOSOMAL LOCATION

Genetic locus: EIF5 (human) mapping to 14q32.32.

PRODUCT

eIF5 siRNA (h) 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 eIF5 shRNA Plasmid (h): sc-35288-SH and eIF5 shRNA (h) Lentiviral Particles: sc-35288-V as alternate gene silencing products.

For independent verification of eIF5 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35288A, sc-35288B and sc-35288C.

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

eIF5 siRNA (h) is recommended for the inhibition of eIF5 expression in human 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

eIF5 (E-10): sc-28309 is recommended as a control antibody for monitoring of eIF5 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 eIF5 gene expression knockdown using RT-PCR Primer: eIF5 (h)-PR: sc-35288-PR (20 µl). 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.