

eIF5B siRNA (h): sc-94706

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. eIF5B (eukaryotic translation initiation factor 5B), also known as translation initiation factor IF-2, is a 1,120 amino acid cytoplasmic protein that functions in general translation initiation by promoting the binding of the formylmethionine-tRNA to ribosomes. eIF5B interacts with Annexin V, an anticoagulant protein, in a calcium and phospholipid-dependent manner. Since eIF5B is conserved among all three kingdoms of life, it is also known as an universal initiation factor.

REFERENCES

1. Roll-Mecak, A., et al. 2001. Engaging the ribosome: universal IFs of translation. *Trends Biochem. Sci.* 26: 705-709.
2. Lee, J.H., et al. 2002. Initiation factor eIF5B catalyzes second GTP-dependent step in eukaryotic translation initiation. *Proc. Natl. Acad. Sci. USA* 99: 16689-16694.
3. Unbehauen, A., et al. 2007. Position of eukaryotic initiation factor eIF5B on the 80S ribosome mapped by directed hydroxyl radical probing. *EMBO J.* 26: 3109-3123.
4. Shin, B.S. and Dever, T.E. 2007. Molecular genetic structure-function analysis of translation initiation factor eIF5B. *Meth. Enzymol.* 429: 185-201.
5. Allen, G.S. and Frank, J. 2007. Structural insights on the translation initiation complex: ghosts of a universal initiation complex. *Mol. Microbiol.* 63: 941-950.
6. Jun, K.O., et al. 2008. Functional equivalence of translation factor eIF5B from *Candida albicans* and *Saccharomyces cerevisiae*. *Mol. Cells* 25: 172-177.
7. de Breynne, S., et al. 2008. Cleavage of eukaryotic initiation factor eIF5B by enterovirus 3C proteases. *Virology* 378: 118-122.

CHROMOSOMAL LOCATION

Genetic locus: EIF5B (human) mapping to 2q11.2.

PRODUCT

eIF5B 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see eIF5B shRNA Plasmid (h): sc-94706-SH and eIF5B shRNA (h) Lentiviral Particles: sc-94706-V as alternate gene silencing products.

For independent verification of eIF5B (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-94706A, sc-94706B and sc-94706C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

eIF5B siRNA (h) is recommended for the inhibition of eIF5B 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 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

eIF5B (D-9): sc-393564 is recommended as a control antibody for monitoring of eIF5B 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 eIF5B gene expression knockdown using RT-PCR Primer: eIF5B (h)-PR: sc-94706-PR (20 μ l, 415 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.