

eIF4AI siRNA (h): sc-40554

BACKGROUND

Translation initiation in eukaryotes necessitates the assembly of an 80S ribosomal complex. Eukaryotic initiation factors (eIFs) are utilized in a sequence of reactions that leads to 80S ribosomal assembly and initiation of translation. Mammalian eukaryotic translation initiation factor 4F (eIF4F) is a protein complex that contains eIF4A, eIF4E and eIF4G, binds mRNA at a 5'-cap motif and recruits the 43S ribosomal preinitiation complex to the eligible transcript. Along with eIF4B, the eIF4F complex mediates the unwinding of mRNA secondary structure to facilitate ribosome association. eIF4E specifically interacts with the 5' cap, eIF4A (I, II) are bidirectional RNA helicases, and eIF4G (I, II) are scaffolding proteins which coordinate eIF4E, eIF4A, eIF3 and the 40S ribosome. Human eIF4AI (eIF4A, DDX2A) is a 406 amino acid protein that is 92.7% homologous to mouse eIF4AI. The promoter region of human eIF4AI contains TATA and CAAT motifs and consensus binding sites to Sp1 and AP2.

REFERENCES

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3. Kukimoto, I., et al. 1997. Characterization of the cloned promoter of the human initiation factor 4AI gene. *Biochem. Biophys. Res. Commun.* 233: 844-847.
4. Imataka, H., et al. 1998. A newly identified N-terminal amino acid sequence of human eIF4G binds poly(A)-binding protein and functions in poly(A)-dependent translation. *EMBO J.* 17: 7480-7489.
5. Gradi, A., et al. 1998. A novel functional human eukaryotic translation initiation factor 4G. *Mol. Cell. Biol.* 18: 334-342.
6. Online Mendelian Inheritance in Man, OMIM[™]. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 602641: World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
7. Gingras, A.C., et al. 1999. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu. Rev. Biochem.* 68: 913-963.

CHROMOSOMAL LOCATION

Genetic locus: EIF4A1 (human) mapping to 17p13.1.

PRODUCT

eIF4AI 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 eIF4AI shRNA Plasmid (h): sc-40554-SH and eIF4AI shRNA (h) Lentiviral Particles: sc-40554-V as alternate gene silencing products.

For independent verification of eIF4AI (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-40554A, sc-40554B and sc-40554C.

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

eIF4AI siRNA (h) is recommended for the inhibition of eIF4AI 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

eIF4AI/II (H-5): sc-377315 is recommended as a control antibody for monitoring of eIF4AI 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 eIF4AI gene expression knockdown using RT-PCR Primer: eIF4AI (h)-PR: sc-40554-PR (20 μ l, 479 bp). 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.