

eIF3 θ siRNA (m): sc-40551

BACKGROUND

The initiation of protein synthesis in eukaryotic cells is regulated by interactions between protein initiation factors and RNA molecules. Eukaryotic initiation factors (eIFs) are utilized in a sequence of reactions that lead to 80S ribosomal assembly and, ultimately, translation. The eukaryotic initiation factor-3 (eIF3) scaffolding structure is the largest of the eIF complexes and includes eIF3 α , eIF3 β , eIF3 δ , eIF3 γ , eIF3 η , eIF3 ϵ , eIF3 θ and eIF3 ζ , all of which function to control the assembly of the 40S ribosomal subunit. Association of eIF3 proteins with the 40S ribosomal subunit stabilizes eIF2-GTP-Met-tRNA^{Met} complex association and mRNA binding, and promotes dissociation of 80S ribosomes into 40S and 60S subunits, thereby promoting the assembly of the pre-initiation complex. Overexpression of eIF3 proteins is common in several cancers, suggesting a role for eIF3 proteins in tumorigenesis.

REFERENCES

1. Valásek, L., et al. 2004. Interactions of eukaryotic translation initiation factor 3 (eIF3) subunit NIP1/c with eIF1 and eIF5 promote preinitiation complex assembly and regulate start codon selection. *Mol. Cell. Biol.* 24: 9437-9455.
2. Peterson, T.R., et al. 2005. eIF3: a connectTOR of S6K1 to the translation preinitiation complex. *Mol. Cell* 20: 655-657.
3. Dong, Z., et al. 2006. Initiation factor eIF3 and regulation of mRNA translation, cell growth, and cancer. *Crit. Rev. Oncol. Hematol.* 59: 169-180.
4. LeFebvre, A.K., et al. 2006. Translation initiation factor eIF4G-1 binds to eIF3 through the eIF3 ϵ subunit. *J. Biol. Chem.* 281: 22917-22932.
5. Hinnebusch, A.G. 2006. eIF3: a versatile scaffold for translation initiation complexes. *Trends Biochem. Sci.* 31: 553-562.
6. Zhang, L., et al. 2007. Individual overexpression of five subunits of human translation initiation factor eIF3 promotes malignant transformation of immortal fibroblast cells. *J. Biol. Chem.* 282: 5790-5800.
7. Masutani, M., et al. 2007. Reconstitution reveals the functional core of mammalian eIF3. *EMBO J.* 26: 3373-3383.
8. Sato, H., et al. 2007. Measles virus N protein inhibits host translation by binding to eIF3-p40. *J. Virol.* 81: 11569-11576.

CHROMOSOMAL LOCATION

Genetic locus: Eif3a (mouse) mapping to 19 D3.

PRODUCT

eIF3 θ 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 eIF3 θ shRNA Plasmid (m): sc-40551-SH and eIF3 θ shRNA (m) Lentiviral Particles: sc-40551-V as alternate gene silencing products.

For independent verification of eIF3 θ (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-40551A, sc-40551B and sc-40551C.

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

eIF3 θ siRNA (m) is recommended for the inhibition of eIF3 θ 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

eIF3 θ (E-1): sc-365789 is recommended as a control antibody for monitoring of eIF3 θ 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 MarkerTM 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 eIF3 θ gene expression knockdown using RT-PCR Primer: eIF3 θ (m)-PR: sc-40551-PR (20 μ l, 540 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.