

## 4E-T siRNA (m): sc-40524

### BACKGROUND

The eukaryotic initiation complex eIF4F exists *in vitro* as a trimeric complex of eIF4G, eIF4E and eIF4A. Together, the complex allows ribosome binding to mRNA by inducing the unwinding of mRNA secondary structures. eIF4E binds to the mRNA "cap" during an early step in the initiation of protein synthesis. eIF4E-transporter (4E-T) is a nucleocytoplasmic protein that facilitates the nuclear import of eIF4E by regulating the formation of a complex between the eIF4E and the importin  $\alpha\beta$  pathway. This interaction between 4E-T and eIF4E occurs through a conserved binding site. In addition to this binding site for eIF4E, 4E-T contains a bipartite nuclear localization signal and two leucine-rich nuclear export signals. The gene encoding for 4E-T maps to human chromosome 22q12.2.

### REFERENCES

1. Rychlik, W., et al. 1987. Amino acid sequence of the mRNA cap-binding protein from human tissues. *Proc. Natl. Acad. Sci. USA* 84: 945-949.
2. Jaramillo, M., et al. 1991. RNA unwinding in translation: assembly of helicase complex intermediates comprising eukaryotic initiation factors eIF-4F and eIF-4B. *Mol. Cell. Biol.* 11: 5992-5997.
3. Scheper, G.C., et al. 1992. Eukaryotic initiation factors-4E and -4F stimulate 5' cap-dependent as well as internal initiation of protein synthesis. *J. Biol. Chem.* 267: 7269-7274.
4. Merrick, W.C. 1994. Eukaryotic protein synthesis: an *in vitro* analysis. *Biochimie* 76: 822-830.
5. Dostie, J., et al. 2000. A novel shuttling protein, 4E-T, mediates the nuclear import of the mRNA 5' cap-binding protein, eIF4E. *EMBO J.* 19: 3142-3156.
6. LocusLink Report (LocusID: 56478). <http://www.ncbi.nlm.nih.gov/LocusLink/>

### CHROMOSOMAL LOCATION

Genetic locus: Eif4enif1 (mouse) mapping to 11 A1.

### PRODUCT

4E-T 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see 4E-T shRNA Plasmid (m): sc-40524-SH and 4E-T shRNA (m) Lentiviral Particles: sc-40524-V as alternate gene silencing products.

For independent verification of 4E-T (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-40524A, sc-40524B and sc-40524C.

### RESEARCH USE

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

### PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

4E-T siRNA (m) is recommended for the inhibition of 4E-T 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  $\mu$ M in 66  $\mu$ 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

4E-T (B-3): sc-393788 is recommended as a control antibody for monitoring of 4E-T 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 4E-T gene expression knockdown using RT-PCR Primer: 4E-T (m)-PR: sc-40524-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.