

eIF4E siRNA (m2): sc-35313

BACKGROUND

The initiation of protein synthesis in eukaryotic cells is regulated by interactions between protein initiation factors and RNA molecules. 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. eIF4A acts as an ATP-dependent RNA helicase. eIF4G acts as a bridge between eIF4E, eIF4A, and the eIF3 complex.

REFERENCES

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4. 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.
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7. Merrick, W.C. 1994. Eukaryotic protein synthesis: an *in vitro* analysis. *Biochimie* 76: 822-883.
8. Lamphear, B.J., et al. 1995. Mapping of functional domains in eukaryotic protein synthesis initiation factor 4G (eIF4G) with picornaviral proteases. Implications for cap-dependent and cap-independent translational initiation. *J. Biol. Chem.* 270: 21975-21983.

CHROMOSOMAL LOCATION

Genetic locus: Eif4e (mouse) mapping to 3 G3.

PRODUCT

eIF4E siRNA (m2) 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 eIF4E shRNA Plasmid (m2): sc-35313-SH and eIF4E shRNA (m2) Lentiviral Particles: sc-35313-V as alternate gene silencing products.

For independent verification of eIF4E (m2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35313A, sc-35313B and sc-35313C.

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

ENX-1 siRNA (m) is recommended for the inhibition of ENX-1 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 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

eIF4E (P-2): sc-9976 is recommended as a control antibody for monitoring of eIF4E 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 eIF4E gene expression knockdown using RT-PCR Primer: eIF4E (m2)-PR: sc-35313-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.

PROTOCOLS

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