SANTA CRUZ BIOTECHNOLOGY, INC.

eIF4E2 siRNA (m): sc-144619

BACKGROUND

The initiation of protein synthesis in eukaryotic cells is regulated by interactions between protein initiation factors and RNA molecules. The eukaryotic initiation factor 4E family (eIF4E) is comprised of three proteins that are involved in the early initiation of protein synthesis. eIF4E2 (eukaryotic translation initiation factor 4E family member 2), also known as 4EHP, IF4e, 4E-LP or EIF4EL3, is a ubiquitously expressed 245 amino acid protein. During early translation events, eIF4E2 recognizes and binds the 7-methylguanosine-containing mRNA cap (a cotranscriptionally added structure that conveys mRNA stability and allows for efficient RNA processing), thus initiating the unwinding of mRNA secondary structures and facilitating mRNA-ribosome binding. eIF4E2 competes with eIF4E (member 1) for cap binding and, upon modification by the ubiquitin-like protein ISG15 (Interferon-induced 15 kDa protein), exhibits increased mRNA cap affinity.

REFERENCES

1. Gao, M., et al. 1998. Cloning and characterization of human eIF4E genes. J. Biol. Chem. 273: 4622-4628.
2. Rom, E., et al. 1998. Cloning and characterization of 4EHP, a novel mammalian eIF4E-related cap-binding protein. J. Biol. Chem. 273: 13104-13109.
3. Tan, N.G., et al. 2003. Human homologue of ariadne promotes the ubiquitylation of translation initiation factor 4E homologous protein, 4EHP. FEBS Lett. 554: 501-504.
4. Joshi, B., et al. 2004. Characterization of mammalian eIF4E-family members. Eur. J. Biochem. 271: 2189-2203.
5. Tee, A.R., et al. 2004. Characterizing the interaction of the mammalian eIF4E-related protein 4EHP with 4E-BP1. FEBS Lett. 564: 58-62.
6. Rosettani, P., et al. 2007. Structures of the human eIF4E homologous protein, h4EHP, in its m7GTP-bound and unliganded forms. J. Mol. Biol. 368: 691-705.
7. Zuberek, J., et al. 2007. Weak binding affinity of human 4EHP for mRNA cap analogs. RNA 13: 691-697.
8. Okumura, F., et al. 2007. ISG15 modification of the eIF4E cognate 4EHP enhances cap structure-binding activity of 4EHP. Genes Dev. 21: 255-260.

CHROMOSOMAL LOCATION

Genetic locus: Eif4e2 (mouse) mapping to 1 D.

PRODUCT

eIF4E2 siRNA (m) is a pool of 2 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 eIF4E2 shRNA Plasmid (m): sc-144619-SH and eIF4E2 shRNA (m) Lentiviral Particles: sc-144619-V as alternate gene silencing products.

For independent verification of eIF4E2 (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-144619A and sc-144619B.

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

eIF4E2 siRNA (m) is recommended for the inhibition of eIF4E2 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

eIF4E2 (YB-18): sc-100731 is recommended as a control antibody for monitoring of eIF4E2 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 eIF4E2 gene expression knockdown using RT-PCR Primer: eIF4E2 (m)-PR: sc-144619-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.