

4E-BP1 siRNA (m): sc-29595

BACKGROUND

The translation of proteins from eukaryotic mRNA is initiated by the multisubunit complex eIF-4F, which associates with the mRNA 5' cap structure. eIF-4E, a component of eIF-4F, is responsible for binding to the 5' cap structure and for the assembly of the eIF-4F complex. The regulatory protein 4E-BP1, also referred to as PHAS-I, inhibits eIF-4E function. Phosphorylation of 4E-BP1 by S6 kinase p70, MAP kinases or PKCs causes the disassociation of 4E-BP1 from eIF-4E, promoting translation. A protein that is functionally related to 4E-BP1, designated 4E-BP2, also associates with eIF-4E.

REFERENCES

1. Lin, T.A., et al. 1994. PHAS-I as a link between mitogen-activated protein kinase and translation initiation. *Science* 266: 653-656.
2. Rau, M., et al. 1996. A reevaluation of the cap-binding protein, eIF4E, as a rate-limiting factor for initiation of translation in reticulocyte lysate. *J. Biol. Chem.* 271: 8983-8990.
3. Whalen, S.G., et al. 1996. Phosphorylation of eIF-4E on serine 209 by protein kinase C is inhibited by the translational repressors, 4E-binding proteins. *J. Biol. Chem.* 271: 11831-11837.
4. Diggle, T.A., et al. 1996. Both Rapamycin-sensitive and -insensitive pathways are involved in the phosphorylation of the initiation factor-4E-binding protein (4E-BP1) in response to Insulin in rat epididymal fat-cells. *Biochem. J.* 316: 447-453.
5. Beretta, L., et al. 1996. Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. *EMBO J.* 15: 658-664.

CHROMOSOMAL LOCATION

Genetic locus: Eif4ebp1 (mouse) mapping to 8 A2.

PRODUCT

4E-BP1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see 4E-BP1 shRNA Plasmid (m): sc-29595-SH and 4E-BP1 shRNA (m) Lentiviral Particles: sc-29595-V as alternate gene silencing products.

For independent verification of 4E-BP1 (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-29595A and sc-29595B.

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

4E-BP1 siRNA (m) is recommended for the inhibition of 4E-BP1 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

4E-BP1 (P-1): sc-9977 is recommended as a control antibody for monitoring of 4E-BP1 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 4E-BP1 gene expression knockdown using RT-PCR Primer: 4E-BP1 (m)-PR: sc-29595-PR (20 μ l, 562 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Goo, C.K., et al. 2012. PTEN/Akt signaling controls mitochondrial respiratory capacity through 4E-BP1. *PLoS ONE* 7: e45806.
2. Maher, P., et al. 2018. Deciphering the pathways that protect from IL-13-mediated potentiation of oxidative stress-induced dopaminergic nerve cell death. *Cytokine* 103: 114-120.

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.