

4E-BP3 siRNA (h): sc-91863

BACKGROUND

The initiation of protein synthesis in eukaryotic cells is regulated by interactions between protein initiation factors and RNA molecules. These interactions are facilitated, in part, by the eukaryotic initiation factor 4 family (eIF4) of proteins that are involved in the early initiation of protein synthesis. 4E-BP3, also known as EIF4EBP3 (eukaryotic translation initiation factor 4E binding protein 3), is a 100 amino acid protein that belongs to the eIF4E-binding protein family. Highly expressed in heart, kidney, pancreas and skeletal muscle and present at lower levels in thymus and brain, 4E-BP3 interacts with eIF4E and, via this interaction, regulates eIF4E activity, specifically by preventing the incorporation of eIF4E into the eIF4 complex. 4E-BP3 is subject to post-translational phosphorylation and is encoded by a gene which maps to human chromosome 5.

REFERENCES

1. Poulin, F., et al. 1998. 4E-BP3, a new member of the eukaryotic initiation factor 4E-binding protein family. *J. Biol. Chem.* 273: 14002-14007.
2. Kleijn, M., et al. 2002. Localisation and regulation of the eIF4E-binding protein 4E-BP3. *FEBS Lett.* 532: 319-323.
3. Tee, A.R., et al. 2002. Caspase cleavage of initiation factor 4E-binding protein 1 yields a dominant inhibitor of cap-dependent translation and reveals a novel regulatory motif. *Mol. Cell. Biol.* 22: 1674-1683.
4. Ferguson, G., et al. 2003. Ser-64 and Ser-111 in PHAS-I are dispensable for Insulin-stimulated dissociation from eIF4E. *J. Biol. Chem.* 278: 47459-47465.
5. Poulin, F., et al. 2003. Gene fusion and overlapping reading frames in the mammalian genes for 4E-BP3 and MASK. *J. Biol. Chem.* 278: 52290-52297.

CHROMOSOMAL LOCATION

Genetic locus: EIF4EBP3 (human) mapping to 5q31.3.

PRODUCT

4E-BP3 siRNA (h) 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 4E-BP3 shRNA Plasmid (h): sc-91863-SH and 4E-BP3 shRNA (h) Lentiviral Particles: sc-91863-V as alternate gene silencing products.

For independent verification of 4E-BP3 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-91863A, sc-91863B and sc-91863C.

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.

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-BP3 siRNA (h) is recommended for the inhibition of 4E-BP3 expression in human 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-BP3 (4-RY9): sc-134232 is recommended as a control antibody for monitoring of 4E-BP3 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-BP3 gene expression knockdown using RT-PCR Primer: 4E-BP3 (h)-PR: sc-91863-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.