

eIF2B ϵ siRNA (h): sc-35278

BACKGROUND

The initiation of protein synthesis in eukaryotic cells is regulated by interactions between protein initiation factors and RNA molecules. The eukaryotic initiation complex eIF2B exists as a five subunit complex composed of eIF2B α , eIF2B β , eIF2B γ , eIF2B δ , and eIF2B ϵ . The eIF2B complex catalyzes the exchange of GDP for GTP on the eIF2 complex, following the interaction of eIF2/GTP with the 40S ribosomal subunit. Guanine nucleotide exchange factor (GEF) activity is exhibited by the eIF2B ϵ subunit alone, but is greater in the presence of all five eIF2B subunits. Phosphorylation of eIF2 inhibits GEF activity of eIF2B, an inhibition that requires the eIF2B α subunit.

REFERENCES

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2. Flowers, K.M., et al. 1995. Structure and sequence of the gene encoding the α subunit of rat translation initiation factor-2B. *Biochim. Biophys. Acta* 1264: 163-167.
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4. Price, N.T., et al. 1996. eIF2B, the guanine nucleotide-exchange factor for eukaryotic initiation factor 2. Sequence conservation between the α , β and δ subunits of eIF2B from mammals and yeast. *Biochem. J.* 318: 637-643.
5. Asuru, A.I., et al. 1996. Cloning and characterization of cDNAs encoding the ϵ subunit of eukaryotic initiation factor-2B from rabbit and human. *Biochim. Biophys. Acta* 1307: 309-317.
6. Webb, B.L. and Proud, C.G. 1997. Eukaryotic initiation factor 2B (eIF2B). *Int. J. Biochem. Cell Biol.* 29: 1127-1131.
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CHROMOSOMAL LOCATION

Genetic locus: EIF2B5 (human) mapping to 3q27.1.

PRODUCT

eIF2B ϵ 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 eIF2B ϵ shRNA Plasmid (h): sc-35278-SH and eIF2B ϵ shRNA (h) Lentiviral Particles: sc-35278-V as alternate gene silencing products.

For independent verification of eIF2B ϵ (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-35278A, sc-35278B and sc-35278C.

RESEARCH USE

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

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

eIF2B ϵ siRNA (h) is recommended for the inhibition of eIF2B ϵ 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

eIF2B ϵ (B-7): sc-55558 is recommended as a control antibody for monitoring of eIF2B ϵ 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 eIF2B ϵ gene expression knockdown using RT-PCR Primer: eIF2B ϵ (h)-PR: sc-35278-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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