SANTA CRUZ BIOTECHNOLOGY, INC.

eIF2Bα siRNA (h): sc-77248

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 eIF2Bα, eIF2Bβ, eIF2Bγ, eIF2Bδ and eIF2Bε. 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 eIF2Bε 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 eIF2Bα subunit. Defects in the gene encoding eIF2Bα are a cause of leukoencephalopathy with vanishing white matter (VWM), a brain disease that is characterized by head trauma and motor deterioration.

REFERENCES

1. van der Knaap, M.S., et al. 2002. Mutations in each of the five subunits of translation initiation factor eIF2B can cause leukoencephalopathy with vanishing white matter. Ann. Neurol. 51: 264-270.
2. van der Knaap, M.S., et al. 2003. eIF2B-related disorders: antenatal onset and involvement of multiple organs. Am. J. Hum. Genet. 73: 1199-1207.
3. Online Mendelian Inheritance in Man, OMIM™. 2003. Johns Hopkins University, Baltimore, MD. MIM Number: 606686. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Ohlenbusch, A., et al. 2005. Identification of ten novel mutations in patients with eIF2B-related disorders. Hum. Mutat. 25: 411.
5. Singh, C.R., et al. 2006. An eIF5/eIF2 complex antagonizes guanine nucleotide exchange by eIF2B during translation initiation. EMBO J. 25: 4537-4546.
6. Tirosh, S., et al. 2007. A role for eukaryotic translation initiation factor 2B (eIF2B) in taste memory consolidation and in thermal control establishment during the critical period for sensory development. Dev. Neurobiol. 67: 728-739.

CHROMOSOMAL LOCATION

Genetic locus: EIF2B1 (human) mapping to 12q24.31.

PRODUCT

eIF2Bα 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see eIF2Bα shRNA Plasmid (h): sc-77248-SH and eIF2Bα shRNA (h) Lentiviral Particles: sc-77248-V as alternate gene silencing products.

For independent verification of eIF2Bα (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-77248A, sc-77248B and sc-77248C.

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 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

eIF2Bα siRNA (h) is recommended for the inhibition of eIF2Bα 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 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

eIF2Bα (C-11): sc-376846 is recommended as a control antibody for monitoring of eIF2Bα 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 eIF2Bα gene expression knockdown using RT-PCR Primer: eIF2Bα (h)-PR: sc-77248-PR (20 µl). 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.