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EF-2 siRNA (h): sc-43541

BACKGROUND

Two elongation factors (EF) EF-Tu and EF-2 participate in the elongation phase during protein biosynthesis on the ribosome and their functional cycles depend on GTP binding and its hydrolysis. EF-Tu (also designated mitochondrial precursor p43) and EF-2 are multidomain GTPases with essential functions in translation, and they both bind to the same site on the ribosome where their low intrinsic GTPase activities are strongly stimulated. EF-Tu plays a central role in the fast and accurate delivery of aminoacyl-tRNAs to the translating ribosome. In addition, EF-Tu protects the aminoester bond against hydrolysis until a correct match between the codon on mRNA and the anticodon on tRNA can be achieved. EF-2 supports the translocation of tRNAs and of mRNAs on the ribosome so that a new codon can be exposed for decoding.

REFERENCES

- Nyborg, J. 1998. Possible evolution of factors involved in protein biosynthesis. *Acta Biochim. Pol.* 45: 883-894.
- Agrawal, R.K., et al. 1998. Visualization of elongation factor G on the *Escherichia coli* 70S ribosome: the mechanism of translocation. *Proc. Natl. Acad. Sci. USA* 95: 6134-6138.
- Kraal, B., et al. 1999. Translational regulation by modifications of the elongation factor Tu. *Folia Microbiol.* 44: 131-141.
- Martemyanov, K.A., et al. 2000. Domain III of elongation factor G from *T. thermophilus* is essential for induction of GTP hydrolysis on the ribosome. *J. Biol. Chem.* 275: 35820-35824.
- Rodnina, M.V., et al. 2000. GTPases mechanisms and functions of translation factors on the ribosome. *Biol. Chem.* 381: 377-387.

CHROMOSOMAL LOCATION

Genetic locus: EEF2 (human) mapping to 19q13.3.

PRODUCT

EF-2 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 EF-2 shRNA Plasmid (h): sc-43541-SH and EF-2 shRNA (h) Lentiviral Particles: sc-43541-V as alternate gene silencing products.

For independent verification of EF-2 (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-43541A, sc-43541B and sc-43541C.

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

EF-2 siRNA (h) is recommended for the inhibition of EF-2 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

EF-2 (C-9): sc-166415 is recommended as a control antibody for monitoring of EF-2 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 EF-2 gene expression knockdown using RT-PCR Primer: EF-2 (h)-PR: sc-43541-PR (20 μ l, 536 bp). 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.