

EF-Tu siRNA (h): sc-105322

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 amino-ester bond against hydrolysis until a correct match between the codon on mRNA and the anti-codon 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

1. Nyborg, J. 1998. Possible evolution of factors involved in protein biosynthesis. *Acta Biochim. Pol.* 45: 883-894.
2. 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.
3. Kraal, B., et al. 1999. Translational regulation by modifications of the elongation factor Tu. *Folia Microbiol.* 44: 131-141.
4. Martemyanov, K.A. and Gudkov, A.T. 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.
5. 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: TUFM (human) mapping to 16p11.2.

PRODUCT

EF-Tu 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-Tu shRNA Plasmid (h): sc-105322-SH and EF-Tu shRNA (h) Lentiviral Particles: sc-105322-V as alternate gene silencing products.

For independent verification of EF-Tu (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-105322A, sc-105322B and sc-105322C.

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

EF-Tu siRNA (h) is recommended for the inhibition of EF-Tu 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-Tu (A-5): sc-393924 is recommended as a control antibody for monitoring of EF-Tu 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-Tu gene expression knockdown using RT-PCR Primer: EF-Tu (h)-PR: sc-105322-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.