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# EF-2 shRNA (h) Lentiviral Particles: sc-43541-V

## 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 Biochem. 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 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see EF-2 siRNA (h): sc-43541 and EF-2 shRNA Plasmid (h): sc-43541-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^{\circ}$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^{\circ}$  C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

EF-2 shRNA (h) Lentiviral Particles is recommended for the inhibition of EF-2 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

EF-2 (F-9): sc-166409 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l, 536 bp). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.