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CPEB shRNA (m2) Lentiviral Particles: sc-44337-V

BACKGROUND

The regulated translation of messenger RNA is essential for cell-cycle progression, establishment of the body plan during early development, and modulation of key activities in the central nervous system. Cytoplasmic polyadenylation, one mechanism of controlling translation, is driven by cytoplasmic polyadenylation element binding protein, CPEB. CPEB is a highly conserved, sequence-specific RNA-binding protein that binds to the cytoplasmic polyadenylation element, thereby modulating translational repression and mRNA localization. Blocking cytoplasmic polyadenylation by interfering with the CPE or CPEB prevents the translational activation and translational repression of mRNAs crucial for oocyte maturation. CPEB is synthesized during oogenesis and stockpiled in the oocyte. CPEB degradation occurs via the proteasome pathway, most likely through ubiquitin-conjugated intermediates.

REFERENCES

1. Stebbins-Boaz, B., et al. 1999. Maskin is a CPEB-associated factor that transiently interacts with eIF-4E. *Mol. Cell* 4: 1017-1027.
2. Luitjens, C., et al. 2000. CPEB proteins control two key steps in spermatogenesis in *C. elegans*. *Genes Dev.* 14: 2596-2609.
3. Groisman, I., et al. 2000. CPEB, maskin, and cyclin B1 mRNA at the mitotic apparatus: implications for local translational control of cell division. *Cell* 103: 435-447.
4. Mendez, R., et al. 2001. Translational control by CPEB: a means to the end. *Nat. Rev. Mol. Cell. Biol.* 2: 521-529.
5. Reverte, C.G., et al. 2001. CPEB degradation during *Xenopus* oocyte maturation requires a PEST domain and the 26S proteasome. *Dev. Biol.* 231: 447-458.

CHROMOSOMAL LOCATION

Genetic locus: Cpeb1 (mouse) mapping to 7 D3.

PRODUCT

CPEB shRNA (m2) 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 μ l frozen stock containing 1.0×10^6 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 CPEB siRNA (m2): sc-44337 and CPEB shRNA Plasmid (m2): sc-44337-SH as alternate gene silencing products.

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.

APPLICATIONS

CPEB shRNA (m2) Lentiviral Particles is recommended for the inhibition of CPEB expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

CPEB (H-300): sc-33193 is recommended as a control antibody for monitoring of CPEB 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CPEB gene expression knockdown using RT-PCR Primer: CPEB (m2)-PR: sc-44337-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

STORAGE

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

PROTOCOLS

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