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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

CPEB siRNA (m2): sc-44337

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 siRNA (m2) 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 CPEB shRNA Plasmid (m2): sc-44337-SH and CPEB shRNA (m2) Lentiviral Particles: sc-44337-V as alternate gene silencing products.

For independent verification of CPEB (m2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44337A, sc-44337B and sc-44337C.

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

CPEB siRNA (m2) is recommended for the inhibition of CPEB expression in mouse 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

CPEB (G-6): sc-514688 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 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 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.