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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) 



CBP80 shRNA (h) Lentiviral Particles: sc-43669-V

BACKGROUND

In eukaryotes, the majority of mRNAs have an m⁷G cap, which is added cotranscriptionally and plays a critical role in many aspects of mRNA metabolism. The effect of the cap on translation is mediated by the initiation factor eIF-4F, whereas the effect on pre-mRNA splicing involves a nuclear complex (CBC). CBC consists of two cap binding proteins CBP20 and CBP80, which mediate the stimulatory functions of the cap in pre-mRNA splicing, 3' end formation and U snRNA export. The genes CBC1 and CBC2 encode CBP80 and CBP20, respectively. CBP80 comprises three domains, each containing a MIF4G domain. CBP20 has an RNAP fold and associates with the second and third domains of CBP80. CBP also plays a role in nonsense-mediated decay (NMD), which eliminates mRNAs, which prematurely terminate translation. CBP80-bound mRNA undergoes a "pioneer" round of translation before CBP80-CBP20 are replaced by eIF4E, and Upf2 and Upf3 proteins.

REFERENCES

1. Izaurralde, E., et al. 1994. A nuclear cap binding protein complex involved in pre-mRNA splicing. *Cell* 78: 657-668.
2. Izaurralde, E., et al. 1995. A cap-binding protein complex mediating U snRNA export. *Nature* 376: 709-712.
3. Das, B., et al. 2000. The role of nuclear cap binding protein CBC1p of yeast in mRNA termination and degradation. *Mol. Cell. Biol.* 20: 2827-2838.
4. McKendrick, L., et al. 2001. Interaction of eukaryotic translation initiation factor 4G with the nuclear cap-binding complex provides a link between nuclear and cytoplasmic functions of the m⁷ guanosine cap. *Mol. Cell. Biol.* 21: 3632-3641.
5. Mazza, C., et al. 2001. Crystal structure of the human nuclear cap binding complex. *Mol. Cell* 8: 383-396.
6. Ishigaki, Y., et al. 2001. Evidence for a pioneer round of mRNA translation: mRNAs subject to nonsense-mediated decay in mammalian cells are bound by CBP80 and CBP20. *Cell* 106: 607-617.

CHROMOSOMAL LOCATION

Genetic locus: NCBP1 (human) mapping to 9q22.33.

PRODUCT

CBP80 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 µl frozen stock containing 1.0 x 10⁶ 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 CBP80 siRNA (h): sc-43669 and CBP80 shRNA Plasmid (h): sc-43669-SH as alternate gene silencing products.

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.

APPLICATIONS

CBP80 shRNA (h) Lentiviral Particles is recommended for the inhibition of CBP80 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

CBP80 (E-7): sc-271304 is recommended as a control antibody for monitoring of CBP80 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 CBP80 gene expression knockdown using RT-PCR Primer: CBP80 (h)-PR: sc-43669-PR (20 µl, 428 bp). 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.

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.

PROTOCOLS

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