SANTA CRUZ BIOTECHNOLOGY, INC.

CBP20 siRNA (m): sc-38250

BACKGROUND

In eukaryotes, the majority of mRNAs have an m^7G 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 m^7 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: Ncbp2 (mouse) mapping to 16 B2.

PRODUCT

CBP20 siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CBP20 shRNA Plasmid (m): sc-38250-SH and CBP20 shRNA (m) Lentiviral Particles: sc-38250-V as alternate gene silencing products.

For independent verification of CBP20 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-38250A, sc-38250B and sc-38250C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CBP20 siRNA (m) is recommended for the inhibition of CBP20 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 µM in 66 µl. 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

CBP20 (B-1): sc-137123 is recommended as a control antibody for monitoring of CBP20 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 CBP20 gene expression knockdown using RT-PCR Primer: CBP20 (m)-PR: sc-38250-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.