SANTA CRUZ BIOTECHNOLOGY, INC.

CstF-77 siRNA (m): sc-142610

BACKGROUND

Polyadenylation of mRNA precursors is a two-step reaction that requires multiple protein factors. The first step, endonucleolytic cleavage of polyadenylation substrates, requires CstF (cleavage stimulation factor), a heterotrimer that is composed of three distinct subunits. Heterotrimeric CstF recognizes GU- and U-rich sequences located downstream of the polyadenylation site on RNA. CstF-77 (cleavage stimulation factor, 77 kDa subunit), also known as CstF3, is one of the three subunits comprising CstF. It can exist as a homodimer and functions as the bridge, directly interacting with the other two CstF subunits, namely CstF-64 and CstF-50. CstF-77 is highly conserved among eukaryotes. It contains an α-helical structure with 11 HAT (half-a-TPR-containing) repeats and is essential for CstF assembly. In addition, CstF-77 is capable of interacting with CPSF1 and FCP1, other factors involved in polyadenylation.

REFERENCES

1. Takagaki, Y., et al. 1990. A multisubunit factor, CstF, is required for polyadenylation of mammalian pre-mRNAs. Genes Dev. 4: 2112-2120.
2. Takagaki, Y., et al. 1997. RNA recognition by the human polyadenylation factor CstF. Mol. Cell. Biol. 17: 3907-3914.
3. Kleiman, F.E., et al. 1999. Functional interaction of BRCA1-associated BARD1 with polyadenylation factor CstF-50. Science 285: 1576-1579.
4. Takagaki, Y., et al. 2000. Complex protein interactions within the human polyadenylation machinery identify a novel component. Mol. Cell. Biol. 20: 1515-1525.
5. Benoit, B., et al. 2002. Chimeric human CstF-77/*Drosophila* suppressor of forked proteins rescue suppressor of forked mutant lethality and mRNA 3' end processing in *Drosophila*. Proc. Natl. Acad. Sci. USA 99: 10593-10598.
6. Pan, Z., et al. 2006. An intronic polyadenylation site in human and mouse CstF-77 genes suggests an evolutionarily conserved regulatory mechanism. Gene 366: 325-334.
7. Bai, Y., et al. 2007. Crystal structure of murine CstF-77: dimeric association and implications for polyadenylation of mRNA precursors. Mol. Cell 25: 863-875.
8. Legrand, P., et al. 2007. The structure of the CstF-77 homodimer provides insights into CstF assembly. Nucleic Acids Res. 35: 4515-4522.

CHROMOSOMAL LOCATION

Genetic locus: Cstf3 (mouse) mapping to 2 E2.

PRODUCT

CstF-77 siRNA (m) is a pool of 2 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 CstF-77 shRNA Plasmid (m): sc-142610-SH and CstF-77 shRNA (m) Lentiviral Particles: sc-142610-V as alternate gene silencing products.

For independent verification of CstF-77 (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-142610A and sc-142610B.

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

CstF-77 siRNA (m) is recommended for the inhibition of CstF-77 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

CstF-77 (G-5): sc-376575 is recommended as a control antibody for monitoring of CstF-77 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 CstF-77 gene expression knockdown using RT-PCR Primer: CstF-77 (m)-PR: sc-142610-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.

PROTOCOLS

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