SANTA CRUZ BIOTECHNOLOGY, INC.

CstF-64T siRNA (m): sc-142609

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. CstF-64 contains an RNA binding domain and is responsible for the RNA binding activity of CstF. CstF-64 is expressed in all somatic cells and in pre- and postmeiotic, but not meiotic, germ cells. However, a large variant of CstF-64, called t CstF-64, is abundantly expressed in meiotic and postmeiotic cells in the testis and to a lesser extent in the brain, and promotes the germ cell pattern of polyadenylation. The gene encoding CstF-64 (designated CSTF2) maps to the X chromosome, whereas t CstF-64 is encoded by an autosomal gene. The increase in CstF-64 concentration during B cell activation switches IgM heavy chain mRNA expression from membrane-bound to secreted forms, suggesting that CstF-64 plays a key role in regulating IgM heavy chain expression during B cell differentiation.

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. 1996. The polyadenylation factor CstF-64 regulates alternative processing of IgM heavy chain pre-mRNA during B cell differentiation. Cell 87: 941-952.
3. Takagaki, Y., et al. 1998. Levels of polyadenylation factor CstF-64 control IgM heavy chain mRNA accumulation and other events associated with B cell differentiation. Mol. Cell 2: 761-771.
4. Kleiman, F.E., et al. 1999. Functional interaction of BRCA1-associated BARD1 with polyadenylation factor CstF-50. Science 285: 1576-1579.
5. Wallace, A.M., et al. 1999. Two distinct forms of the 64,000 Mr protein of the cleavage stimulation factor are expressed in mouse male germ cells. Proc. Natl. Acad. Sci. USA 96: 6763-6768.
6. Takagaki, Y., et al. 2000. Complex protein interactions within the human polyadenylation machinery identify a novel component. Mol. Cell. Biol. 20: 1515-1525.

CHROMOSOMAL LOCATION

Genetic locus: Cstf2t (mouse) mapping to 19 C1.

PRODUCT

CstF-64T 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-64T shRNA Plasmid (m): sc-142609-SH and CstF-64T shRNA (m) Lentiviral Particles: sc-142609-V as alternate gene silencing products.

For independent verification of CstF-64T (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-142609A and sc-142609B.

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-64T siRNA (m) is recommended for the inhibition of CstF-64T 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CstF-64T gene expression knockdown using RT-PCR Primer: CstF-64T (m)-PR: sc-142609-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.