



CstF-64 siRNA (h): sc-35118

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. and Manley, J.L. 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. and Manley, J.L. 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 M_r protein of the cleavage stimulation factor are expressed in mouse male germ cells. *Proc. Natl. Acad. Sci. USA* 96: 6763-6768.
6. Takagaki, Y. and Manley, J.L. 2000. Complex protein interactions within the human polyadenylation machinery identify a novel component. *Mol. Cell. Biol.* 20: 1515-1525.

CHROMOSOMAL LOCATION

Genetic locus: CSTF2 (human) mapping to Xq22.1.

PRODUCT

CstF-64 siRNA (h) 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 CstF-64 shRNA Plasmid (h): sc-35118-SH and CstF-64 shRNA (h) Lentiviral Particles: sc-35118-V as alternate gene silencing products.

For independent verification of CstF-64 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35118A, sc-35118B and sc-35118C.

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

CstF-64 shRNA Plasmid (h) is recommended for the inhibition of CstF-64 expression in human 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

CstF-64 (B-3): sc-166647 is recommended as a control antibody for monitoring of CstF-64 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 CstF-64 gene expression knockdown using RT-PCR Primer: CstF-64 (h)-PR: sc-35118-PR (20 μ l, 419 bp). 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.