

CstF-50 siRNA (m): sc-37754

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. The shortest CstF subunit shares extensive homology with mammalian G protein β -subunits and has a transducin repeat domain, which is a 44-amino acid-long sequence that is repeated seven times. CstF-50 interacts with the nuclear protein BARD1 (BRCA1-associated RING domain protein) and inhibits polyadenylation *in vitro*. CstF-50 may also be responsible for the interaction of the heterotrimeric CstF complex with other polyadenylation and 3'-end cleavage factors to form a stable complex on the pre-mRNA.

REFERENCES

1. Takagaki, Y., Manley, J.L., MacDonald, C.C., Wilusz, J. and Shenk, T. 1990. A multisubunit factor, CstF, is required for polyadenylation of mammalian pre-mRNAs. *Genes Dev.* 4: 2112-2120.
2. Takagaki, Y. and Manley, J.L. 1992. A human polyadenylation factor is a G protein β -subunit homologue. *J. Biol. Chem.* 267: 23471-23474.
3. Takagaki, Y. and Manley, J.L. 1997. RNA recognition by the human polyadenylation factor CstF. *Mol. Cell. Biol.* 17: 3907-3914.
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. 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: Cstf1 (mouse) mapping to 2 H3.

PRODUCT

CstF-50 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CstF-50 shRNA Plasmid (m): sc-37754-SH and CstF-50 shRNA (m) Lentiviral Particles: sc-37754-V as alternate gene silencing products.

For independent verification of CstF-50 (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-37754A, sc-37754B and sc-37754C.

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.

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-50 siRNA (m) is recommended for the inhibition of CstF-50 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 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-50 (A-5): sc-393260 is recommended as a control antibody for monitoring of CstF-50 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-50 gene expression knockdown using RT-PCR Primer: CstF-50 (m)-PR: sc-37754-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.