SANTA CRUZ BIOTECHNOLOGY, INC.

CPSF1 siRNA (m): sc-35102

BACKGROUND

Processing of the 3' end of mRNA depends on several protein factors, one of which is the cleavage and polyadenylation specificity factor (CPSF). CPSF is required for both the cleavage of the mRNA precursor and for polyadenylation. CPSF, a multisubunit factor consisting of four subunits, is localized to the nucleoplasm and is excluded from cytoplasmic and nucleolar structures in HeLa cells. CPSF recognizes the AAUAAA signal in the pre-mRNA and interacts with other proteins to facilitate both RNA cleavage and poly(A) synthesis. The largest subunit of CPSF can, by itself, bind preferentially to AAUAAA-containing RNAs and binds specifically to both the suppressor of forked subunit of the cleavage stimulatory factor (CstF) and to poly (A) polymerase. U1 snRNP-A protein (U1A) interacts with and affects the activity of CPSF by stabilizing the interaction of CPSF with the AAUAAA-containing RNAs to increase the efficiency of polyadenylation. Efficient processing of 3' core poly(A) site also requires sequences 76 nucleotides upstream of the AAUAA hexamer. The largest subunit is able to interact directly with the HIV-1 upstream element to direct a stable binding of CPSF to the pre-mRNA and enhance the efficiency of polyadenylation.

REFERENCES

1. Jenny, A., et al. 1994. Characterization of cleavage and polyadenylation specificity factor and cloning of its 100 kDa subunit. Mol. Cell. Biol. 14: 8183-8190.
2. Gilmartin, G.M., et al. 1995. CPSF recognition of an HIV-1 mRNA 3'-processing enhancer: multiple sequence contacts involved in poly(A) site definition. Genes Dev. 9: 72-83.
3. Murthy, K.G., et al. 1995. The 160 kDa subunit of human cleavage-polyadenylation specificity factor coordinates pre-mRNA 3'-end formation. Genes Dev. 9: 2672-2683.
4. Jenny, A., et al. 1995. Cloning and cDNAs encoding the 160 kDa subunit of the bovinne cleavage and polyadenylation specificity factor. Nucleic Acids Res. 23: 2629-2635.
5. Lutz, C.S., et al. 1996. Interaction between the U1 snRNP-A protein and the 160 kDa subunit of cleavage-polyadenylation specificity factor increases polyadenylation efficiency *in vitro*. Genes Dev. 10: 325-337.

CHROMOSOMAL LOCATION

Genetic locus: Cpsf1 (mouse) mapping to 15 D3.

PRODUCT

CPSF1 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 CPSF1 shRNA Plasmid (m): sc-35102-SH and CPSF1 shRNA (m) Lentiviral Particles: sc-35102-V as alternate gene silencing products.

For independent verification of CPSF1 (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-35102A, sc-35102B and sc-35102C.

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

CPSF1 shRNA Plasmid (m) is recommended for the inhibition of CPSF1 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

CPSF1 (G-10): sc-166281 is recommended as a control antibody for monitoring of CPSF1 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 CPSF1 gene expression knockdown using RT-PCR Primer: CPSF1 (m)-PR: sc-35102-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.