

CPSF4 siRNA (h): sc-72988

BACKGROUND

CPSF4 (cleavage and polyadenylation specificity factor subunit 4, NS1 effector domain-binding protein 1) is a nuclear protein that belongs to the CPSF4/YTH1 family and contains five C₂H₁-type zinc fingers and one CCHC-type zinc finger. CPSF4 is a component of the cleavage and polyadenylation specificity factor (CPSF) complex that plays a key role in pre-mRNA 3'-end formation. CPSF is a multi-subunit factor consisting of four subunits. 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. snRNP protein (U1 snRNP-A) 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 specific sequences located 76 nucleotides upstream of the AAUAAA hexamer.

REFERENCES

1. Jenny, A., et al. 1995. Cloning and cDNAs encoding the 160 kDa subunit of the bovine cleavage and polyadenylation specificity factor. *Nucleic Acids Res.* 23: 2629-2635.
2. Barabino, S.M., et al. 1997. The 30-kD subunit of mammalian cleavage and polyadenylation specificity factor and its yeast homolog are RNA-binding zinc finger proteins. *Genes Dev.* 11: 1703-1716.
3. Salinas, C.A., et al. 1998. Characterization of a *Drosophila* homologue of the 160-kDa subunit of the cleavage and polyadenylation specificity factor CPSF. *Mol. Gen. Genet.* 257: 672-680.
4. de Vries, H., et al. 2000. Human pre-mRNA cleavage factor II_m contains homologs of yeast proteins and bridges two other cleavage factors. *EMBO J.* 19: 5895-5904.

CHROMOSOMAL LOCATION

Genetic locus: CPSF4 (human) mapping to 7q22.1.

PRODUCT

CPSF4 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 CPSF4 shRNA Plasmid (h): sc-72988-SH and CPSF4 shRNA (h) Lentiviral Particles: sc-72988-V as alternate gene silencing products.

For independent verification of CPSF4 (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-72988A, sc-72988B and sc-72988C.

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

CPSF4 siRNA (h) is recommended for the inhibition of CPSF4 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

CPSF4 (A-11): sc-393316 is recommended as a control antibody for monitoring of CPSF4 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 CPSF4 gene expression knockdown using RT-PCR Primer: CPSF4 (h)-PR: sc-72988-PR (20 μ l, 537 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.