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FCP1 siRNA (h): sc-44888

BACKGROUND

RNA polymerase II (RNAP II) comprises a 12 subunit protein complex that mediates transcription. RNAP II contains a unique carboxy terminal domain (CTD), which consists of 52 repeats of the consensus heptapeptide Tyr-Ser-Pro-Thr-Ser-Pro-Ser. The RNAP II isoform with an unphosphorylated CTD associates with transcription initiation complexes, whereas the isoform with a phosphorylated CTD is involved in transcription elongation. FCP1, also designated TFIIIF-associated CTD phosphatase, is a protein phosphatase dedicated to the CTD of RNAP II that mediates the recycling of RNAP II during the transcription cycle. FCP1 specifically targets phosphorylated CTD Serine 2 and 5 with similar affinities. Phosphorylation of FCP1 itself is necessary for sufficient activity of the protein and its interaction with TFIIIF. Upon activation, the α -helical carboxy terminus of FCP1 binds to Rap 74 to form a complex. FCP1 also represses HIV-1 TAT-mediated transactivation and, therefore, may represent a specific target for modulation of TAT activity in infected cells.

REFERENCES

1. Licciardo, P., Ruggiero, L., Lania, L. and Majello, B. 2001. Transcription activation by targeted recruitment of the RNA polymerase II CTD phosphatase FCP1. *Nucleic Acids Res.* 29: 3539-3545.
2. Licciardo, P., Napolitano, G., Majello, B. and Lania, L. 2001. Inhibition of TAT transactivation by the RNA polymerase II CTD-phosphatase FCP1. *AIDS* 15: 301-307.
3. Mandal, S.S., Cho, H., Kim, S., Cabane, K. and Reinberg, D. 2002. FCP1, a phosphatase specific for the heptapeptide repeat of the largest subunit of RNA polymerase II, stimulates transcription elongation. *Mol. Cell. Biol.* 22: 7543-7552.
4. Lin, P.S., Dubois, M.F. and Dahmus, M.E. 2002. TFIIIF-associating carboxyl-terminal domain phosphatase dephosphorylates phosphoserines 2 and 5 of RNA polymerase II. *J. Biol. Chem.* 277: 45949-45956.

CHROMOSOMAL LOCATION

Genetic locus: CTD1P1 (human) mapping to 18q23.

PRODUCT

FCP1 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 FCP1 shRNA Plasmid (h): sc-44888-SH and FCP1 shRNA (h) Lentiviral Particles: sc-44888-V as alternate gene silencing products.

For independent verification of FCP1 (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-44888A, sc-44888B and sc-44888C.

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

FCP1 siRNA (h) is recommended for the inhibition of FCP1 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

FCP1 (3G4): sc-293358 is recommended as a control antibody for monitoring of FCP1 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 FCP1 gene expression knockdown using RT-PCR Primer: FCP1 (h)-PR: sc-44888-PR (20 μ l). 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.