SANTA CRUZ BIOTECHNOLOGY, INC.

cyclin T1 siRNA (m): sc-35144

BACKGROUND

Cyclin T1 was identified as a partner for Cdk9, an RNA polymerase II (RNAPII) transcription elongation factor. Cyclin T1 interacts with the *trans*-activation domain of the HIV-1 Tat protein. The interaction of Tat with cyclin T1 enhances the affinity of Tat for the viral TAR RNA stem-loop structure, suggesting that Tat can recruit cyclin T1/Cdk9 to RNAPII through cooperative binding to TAR. The human positive transcription elongation factor b (P-TEFb) consists of a cyclin dependent kinase, Cdk9, paired with a cyclin T. Cdk9 may be paired with either cyclin T1 or cyclin T2, in a mutually exclusive manner. Two forms of cyclin T2, T2a and T2b, are due to alternative splicing. The binding of Tat to TAR was shown to be facilitated by human cyclin T1, but not by cyclins T2a or T2b. Cyclin T2 binds to Cdk9 but not to Tat, and cyclin T2 can inhibit cyclin T1-mediated Tat activity.

REFERENCES

1. Herrmann, C.H. and Rice, A.P. 1995. Lentivirus TAT proteins specifically associate with a cellular protein kinase, TAK, that hyperphosphorylates the carboxyl-terminal domain of the large subunit of RNA polymerase II: candidate for a TAT cofactor. J. Virol. 69: 1612-1620.
2. Yang, X., et al. 1997. TAK, an HIV TAT-associated kinase, is a member of the cyclin-dependent family of protein kinases and is induced by activation of peripheral blood lymphocytes and differentiation of promonocytic cell lines. Proc. Natl. Acad. Sci. USA 94: 12331-12336.
3. Wei, P., et al. 1998. A novel Cdk9-associated C-type cyclin interacts directly with HIV-1 TAT and mediates its high-affinity, loop-specific binding to TAR RNA. Cell 92: 451-462.
4. Peng, J., et al. 1998. Identification of multiple cyclin subunits of human P-TEFb. Genes Dev. 12: 755-762.
5. Wimmer, J., et al. 1999. Interactions between Tat and TAR and human immunodeficiency virus replication are facilitated by human cyclin T1 but not cyclins T2a or T2b. Virology 255: 182-189.
6. Napolitano, G., et al. 1999. The CDK9-associated cyclins T1 and T2 exert opposite effects on HIV-1 Tat activity. AIDS 13: 1453-1459.

CHROMOSOMAL LOCATION

Genetic locus: Ccnt1 (mouse) mapping to 15 F1.

PRODUCT

cyclin T1 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 cyclin T1 shRNA Plasmid (m): sc-35144-SH and cyclin T1 shRNA (m) Lentiviral Particles: sc-35144-V as alternate gene silencing products.

For independent verification of cyclin T1 (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-35144A, sc-35144B and sc-35144C.

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

cyclin T1 shRNA (m) Lentiviral Particles is recommended for the inhibition of cyclin T1 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

cyclin T1 (C-6): sc-271575 is recommended as a control antibody for monitoring of cyclin T1 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 cyclin T1 gene expression knockdown using RT-PCR Primer: cyclin T1 (m)-PR: sc-35144-PR (20 µl, 566 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.