SANTA CRUZ BIOTECHNOLOGY, INC.

TOK-1 siRNA (h): sc-106626

BACKGROUND

Combinations of cyclin-cyclin-dependent kinase (CDK) complex and their inhibitors coordinately regulate cell-cycle movement. INK4 family proteins p15, p16, p18 and P19 inhibit CDK4/CDK, whereas Cip/Kip family proteins p21, p27 and P57, inhibit all of the CDKs. p21 induces cell cycle arrest, thus inhibiting CDK activity for Rb inactivation. In addition to binding of CDK-cyclin to the N-terminal region of p21, other proteins such as proliferating cell nuclear antigen (PCNA), SET/TAF1 and calmodulin are able to bind to the C-proximal region of p21. A novel $p21^{Cip1}$-binding protein TOK-1 binds to the C-terminal region of p21. TOK-1 is alternatively spliced to form TOK-1α and TOK1β, which are comprised of 322 and 314 amino acids, respectively. TOK-1 co-localizes with p21 in nuclei and has similiar expression pattern to that of p21. TOK1α, but not TOK-1β, directly binds to the C-terminal proximal region of p21 and both are expressed at the G_1/S boundary of cell-cycle. TOK-1α preferentially binds to an active form of CDK2 via p21 to make a ternary complex in human cells. In addition, TOK-1α enhances the inhibitory activity of p21 to Histone H1 kinase activity of CDK2, suggesting that TOK-1α may be a new type of CDK2 modulator.

REFERENCES

1. Chen, J., et al. 1995. Separate domains of p21 involved in the inhibition of Cdk kinase and PCNA. Nature 374: 386-388.
2. Goubin F. and Ducommun B. 1995. Identification of binding domains on the $p21^{Cip1}$ cyclin-dependent kinase inhibitor. Oncogene 10: 2281-2287.
3. Harper, J.W., et al. 1995. Inhibition of cyclin-dependent kinases by p21 Mol. Biol. Cell 6: 387-400.
4. Luo, Y., et al. 1995. Cell-cycle inhibition by independent CDK and PCNA binding domains in $p21^{Cip1}$. Nature 375: 159-161.
5. Connell-Crowley, L., et al. 1998. G_1 cyclin-dependent kinases are sufficient to initiate DNA synthesis in quiescent human fibroblasts. Curr. Biol. 8: 65-68.
6. Hengstschlager, M., et al. 1999. Cyclin-dependent kinases at the G_1-S transition of the mammalian cell cycle. Mutat. Res. 436: 1-9.
7. Ono, T., et al. 2000. TOK-1, novel $p21^{Cip1}$-binding protein that cooperatively enhances p21-dependent inhibitory activity toward CDK2 kinase. J. Biol. Chem. 275: 31145-31154.

CHROMOSOMAL LOCATION

Genetic locus: BCCIP (human) mapping to 10q26.2.

PRODUCT

TOK-1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TOK-1 shRNA Plasmid (h): sc-106626-SH and TOK-1 shRNA (h) Lentiviral Particles: sc-106626-V as alternate gene silencing products.

For independent verification of TOK-1 (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-106626A, sc-106626B and sc-106626C.

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

TOK-1 siRNA (h) is recommended for the inhibition of TOK-1 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 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

TOK-1β (B-10): sc-271985 is recommended as a control antibody for monitoring of TOK-1 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TOK-1 gene expression knockdown using RT-PCR Primer: TOK-1 (h)-PR: sc-106626-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Dixit, U., et al. 2014. Fuse binding protein antagonizes the transcription activity of tumor suppressor protein p53. BMC Cancer 14: 925.

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.