

TOK-1 siRNA (m): sc-154549

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 p21Cip1-binding protein TOK-1 binds to the C-terminal region of p21. TOK-1 is alternatively spliced to form TOK-1a and TOK-1b, which are comprised of 322 and 314 amino acids, respectively. TOK-1 co-localizes with p21 in nuclei and has similar expression pattern to that of p21. TOK-1a, but not TOK-1b, directly binds to the C-terminal proximal region of p21 and both are expressed at the G₁/S boundary of cell-cycle. TOK-1a preferentially binds to an active form of CDK2 via p21 to make a ternary complex in human cells. In addition, TOK-1a enhances the inhibitory activity of p21 to histone H1 kinase activity of CDK2, suggesting that TOK-1a may be a new type of CDK2 modulator.

REFERENCES

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CHROMOSOMAL LOCATION

Genetic locus: Bccip (mouse) mapping to 7 F3.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

PRODUCT

TOK-1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TOK-1 shRNA Plasmid (m): sc-154549-SH and TOK-1 shRNA (m) Lentiviral Particles: sc-154549-V as alternate gene silencing products.

For independent verification of TOK-1 (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-154549A, sc-154549B and sc-154549C.

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

TOK-1 siRNA (m) is recommended for the inhibition of TOK-1 expression in mouse cells.

SUPPORT REAGENTS

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.

GENE EXPRESSION MONITORING

TOK-1 β (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 (m)-PR: sc-154549-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.