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TOK-1 siRNA (m): sc-154549



The Power to Question

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 CDKcyclin 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 rgeion of p21. TOK-1 is alternatively spliced to form TOK-1a and TOK1b, 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. TOK1a, 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.

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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 μM 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-lgG κ BP-HRP: sc-516102 or m-lgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgG κ BP-FITC: sc-516140 or m-lgG 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-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 (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.

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