

Wee 1 siRNA (h): sc-36835

BACKGROUND

Phosphorylation of Cdc2 on Threonine 14 and Tyrosine 15 is required to maintain Cdc2 in an inactive state throughout the S and G₂ phases of the cell cycle. The human Wee 1 protein, WEE1Hu, encodes a tyrosine-specific protein kinase that phosphorylates Cdc2 on tyrosine 15. Myt 1, a member of the Wee 1 family of protein kinases, has been shown to phosphorylate Cdc2 on both Threonine 14 and Tyrosine 15 in a cyclin-dependent manner. Activity of both Wee 1 Hu and Myt 1 is regulated during the cell cycle, suggesting that both proteins play a role in mitotic control. Dephosphorylation of Cdc2 on Threonine 14 and Tyrosine 15 in late G₂ by Cdc25 then activates the Cdc2/cyclin B complex to allow entry into mitosis.

REFERENCES

1. Morla, A., et al. 1989. Reversible tyrosine phosphorylation of Cdc2: dephosphorylation accompanies activation during entry into mitosis. *Cell* 58: 193-203.
2. Krek, W., et al. 1991. Differential phosphorylation of vertebrate p34Cdc2 kinase at the G₁/S and G₂/M transitions of the cell cycle: identification of major phosphorylation sites. *EMBO J.* 10: 305-316.
3. Igarashi, M., et al. 1991. Wee 1-like gene in human cells. *Nature* 353: 80-83.
4. McGowan, C.H., et al. 1995. Human Wee 1 kinase inhibits cell division by phosphorylating p34Cdc2 exclusively on Tyr 15. *EMBO J.* 12: 75-85.
5. Watanabe, N., et al. 1995. Regulation of the human WEE1Hu Cdk Tyrosine 15 kinase during the cell cycle. *EMBO J.* 14: 1878-1891.

CHROMOSOMAL LOCATION

Genetic locus: WEE1 (human) mapping to 11p15.4.

PRODUCT

Wee 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Wee 1 shRNA Plasmid (h): sc-36835-SH and Wee 1 shRNA (h) Lentiviral Particles: sc-36835-V as alternate gene silencing products.

For independent verification of Wee 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-36835A, sc-36835B and sc-36835C.

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

Wee 1 siRNA (h) is recommended for the inhibition of Wee 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 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

Wee 1 (B-11): sc-5285 is recommended as a control antibody for monitoring of Wee 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).

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 Wee 1 gene expression knockdown using RT-PCR Primer: Wee 1 (h)-PR: sc-36835-PR (20 μ l, 506 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ambrosini, G., et al. 2015. BRD4-targeted therapy induces Myc-independent cytotoxicity in Gnaq/11-mutant uveal melanoma cells. *Oncotarget* 6: 33397-33409.

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.