SANTA CRUZ BIOTECHNOLOGY, INC.

Myt 1 siRNA (h): sc-35997

BACKGROUND

Phosphorylation of Cdc2 on threonine 14 and tyrosine 15 is required to maintain Cdc2 in an inactive state throughout the S and G_2 phases of the cell cycle. The human Wee 1 protein, Wee 1 Hu, 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 G_2 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 $p34^{cdc2}$ kinase at the G_1/S and G_2/M transitions of the cell cycle: identification of major phosphorylation sites. EMBO J. 10: 305-316.
3. Strausfeld, U., et al. 1991. Dephosphorylation and activation of a $p34^{cdc2}$/cyclin B complex *in vitro* by human CDC25 protein. Nature 351: 242-245.
4. Igarashi, M., et al. 1991. Wee 1^{+}-like gene in human cells. Nature 353: 80-83.
5. Gautier, J., et al. 1991. Cdc25 is a specific tyrosine phosphatase that directly activates $p34^{cdc2}$. Cell 67: 197-211
6. McGowan, C.H., et al. 1995. Human Wee 1 kinase inhibits cell division by phosphorylating $p34^{cdc2}$ exclusively on Tyr15. EMBO J. 12: 75-85.
7. Watanabe, N., et al. 1995. Regulation of the human Wee 1 Hu Cdk tyrosine 15-kinase during the cell cycle. EMBO J. 14: 1878-1891.
8. Liu, F., et al. 1997. The human Myt1 kinase preferentially phosphorylates Cdc2 on threonine 14 and localizes to the endoplasmic reticulum and Golgi complex. Mol. Cell. Biol. 17: 571-583.

CHROMOSOMAL LOCATION

Genetic locus: PKMYT1 (human) mapping to 16p13.3.

PRODUCT

Myt 1 siRNA (h) is a pool of 2 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 Myt 1 shRNA Plasmid (h): sc-35997-SH and Myt 1 shRNA (h) Lentiviral Particles: sc-35997-V as alternate gene silencing products.

For independent verification of Myt 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-35997A and sc-35997B.

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

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

Myt 1 (G-11): sc-74523 is recommended as a control antibody for monitoring of Myt 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-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 Myt 1 gene expression knockdown using RT-PCR Primer: Myt 1 (h)-PR: sc-35997-PR (20 µl, 446 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.