SANTA CRUZ BIOTECHNOLOGY, INC.

Mat1 siRNA (m): sc-35862

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Progression through the cell cycle requires activation of a series of enzymes designated cyclin dependent kinases (Cdks). The monomeric catalytic subunit, Cdk2, a critical enzyme for initiation of cell cycle progression, is completely inactive. Partial activation is achieved by the binding of regulatory cyclins such as cyclin D1, while full activation requires phosphorylation at Thr 160. The enzyme responsible for phosphorylation of Thr 160 in Cdk2 and also Thr 161 in Cdc2 p34, designated Cdk-activating kinase (CAK), has been partially purified and shown to be comprised of a catalytic subunit, a regulatory subunit and a subunit of unknown function. The regulatory subunit is a novel cyclin (cyclin H) and is required for activation of Cdk7. This previously undescribed protein, now termed Mat1, has been cloned as a protein that associates with the cyclin H-Cdk7 nuclear complex at all stages of the cell cycle. Cyclin H-Cdk7-Mat1 complexes display kinase activity towards Cdk activation domains, and the carboxy terminus of RNA polymerase II. Mat1 appears to constitute the first example of an assembly factor, essential for the formation of an active Cdk-cyclin complex.

REFERENCES

1. Nurse, P. 1994. Ordering S phase and M phase in the cell cycle. Cell 79: 547-550.
2. Sherr, C.J. 1994. G_1 phase progression: cycling on cue. Cell 79: 551-555.
3. Hunter, T., et al. 1994. Cyclins and cancer II: cyclin D and Cdk inhibitors come of age. Cell 79: 573-582.
4. Kato, J.Y., et al. 1994. Regulation of cyclin D-dependent kinase 4 (Cdk4) by Cdk4-activating kinase. Mol. Cell. Biol. 14: 2713-2721.
5. Matsuoka, M., et al. 1994. Activation of cyclin-dependent kinase 4 (Cdk4) by mouse MO15-associated kinase. Mol. Cell. Biol. 14: 7265-7275.
6. Fisher, R.P., et al. 1995. Alternative mechanisms of CAK assembly require an assembly factor or an activating kinase. Cell 83: 47-57.
7. Yee, A., et al. 1995. Molecular cloning of Cdk7-associated human Mat1, a cyclin-dependent kinase-activating kinase (CAK) assembly factor. Cancer Res. 55: 6058-6062.

CHROMOSOMAL LOCATION

Genetic locus: Mnat1 (mouse) mapping to 12 C3.

PRODUCT

Mat1 siRNA (m) 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 Mat1 shRNA Plasmid (m): sc-35862-SH and Mat1 shRNA (m) Lentiviral Particles: sc-35862-V as alternate gene silencing products.

For independent verification of Mat1 (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-35862A and sc-35862B.

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

Mat1 siRNA (m) is recommended for the inhibition of Mat1 expression in mouse 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

Mat1 (F-6): sc-13142 is recommended as a control antibody for monitoring of Mat1 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 Mat1 gene expression knockdown using RT-PCR Primer: Mat1 (m)-PR: sc-35862-PR (20 µl, 597 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.