

MAD1 siRNA (m): sc-62578

BACKGROUND

Cell cycle progression is subject to arrest at the mitotic spindle assembly checkpoint in response to incorrect spindle fiber assembly. MAD1 and MAD2 (for mitotic arrest-deficient 1 and 2) are components of the mitotic spindle checkpoint. Incorrect spindle assembly in normal cells leads to mitotic arrest. MAD1 prevents the onset of anaphase until all chromosomes are aligned correctly at the metaphase plate and is crucial for anchoring MAD2L1 to the nuclear periphery. It also plays an important role in septum positioning. MAD1 can form a homo-dimer, but may also form a heterodimer with MAD2 to form the tetrameric MAD1L1-MAD2L1 core complex. MAD1 localizes primarily to the nucleus, but during mitosis, it moves from a nuclear distribution to the centrosome, to the spindle midzone and then on to the midbody. MAD1 activity is induced by BUB1 and the protein is hyperphosphorylated after mitotic spindle damage and/or in late S through M phase. Defects in the gene encoding for MAD1, MAD1L1, play a major role in the development and progression of various cancer types.

REFERENCES

1. Jones, N. 1990. Transcriptional regulation by dimerization: two sides to an incestuous relationship. *Cell* 61: 9-11.
2. Prendergast, G.C., et al. 1991. Association of Myn, the murine homolog of Max, with c-Myc stimulates methylation-sensitive DNA binding and Ras cotransformation. *Cell* 65: 395-407.
3. Blackwood, E.M., et al. 1991. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science* 251: 1211-1217.

CHROMOSOMAL LOCATION

Genetic locus: Mad1l1 (mouse) mapping to 5 G2.

PRODUCT

MAD1 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 MAD1 shRNA Plasmid (m): sc-62578-SH and MAD1 shRNA (m) Lentiviral Particles: sc-62578-V as alternate gene silencing products.

For independent verification of MAD1 (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-62578A, sc-62578B and sc-62578C.

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

MAD1 siRNA (m) is recommended for the inhibition of MAD1 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 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

MAD1 (F-7): sc-376613 is recommended as a control antibody for monitoring of MAD1 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 MAD1 gene expression knockdown using RT-PCR Primer: MAD1 (m)-PR: sc-62578-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Gulluni, F., et al. 2017. Mitotic spindle assembly and genomic stability in breast cancer require PI3K-C2 α scaffolding function. *Cancer Cell* 32: 444-459.

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.