

MAD2B siRNA (h): sc-106795

BACKGROUND

Cell cycle progression is subject to arrest at the mitotic spindle assembly checkpoint in response to incorrect spindle fiber assembly. MAD2 (for mitotic arrest-deficient) is a component of the mitotic spindle checkpoint. Cells with mutated MAD2 do not undergo mitotic arrest in response to incorrect spindle fiber assembly, which results in missegregation and eventual cell death. A breast carcinoma cell line with reduced MAD2 expression, T-47D, was shown to complete mitosis in the presence of Nocodazole, an inhibitor of mitotic spindle assembly. MAD2 is localized to unattached kinetochores during pro-metaphase and disassociates upon spindle fiber attachment, indicating that MAD2 regulates kinetochore binding to the spindle fibers. Human MAD2 has also been shown to associate with Insulin receptor (IR), but not IGF-IR, implicating MAD2 as a mediator for IR-specific signaling. MAD2B, a MAD2 homolog, is required for the execution of the mitotic checkpoint monitoring the kinetochore-spindle attachment process and, if the process is not complete, MAD2B delays the onset of anaphase.

REFERENCES

1. Murray, A.W. 1992. Creative blocks: cell-cycle checkpoints and feedback controls. *Nature* 359: 599-604.
2. Glotzer, M. 1996. Mitosis: don't get mad, get even. *Curr. Biol.* 6: 1592-1594.
3. Chen, R.H., et al. 1996. Association of spindle assembly checkpoint component XMad2 with unattached kinetochores. *Science* 274: 242-246.
4. Li, Y., et al. 1996. Identification of a human mitotic checkpoint gene: hSMAD2. *Science* 274: 246-248.
5. O'Neill, T.J., et al. 1997. Interaction of MAD2 with the carboxyl-terminus of the Insulin receptor but not with the IGF-IR. Evidence for release from the Insulin receptor after activation. *J. Biol. Chem.* 272: 10035-10040.
6. SWISS-PROT/TrEMBL (Q9UI95). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: MAD2L2 (human) mapping to 1p36.22.

PRODUCT

MAD2B 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 MAD2B shRNA Plasmid (h): sc-106795-SH and MAD2B shRNA (h) Lentiviral Particles: sc-106795-V as alternate gene silencing products.

For independent verification of MAD2B (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-106795A, sc-106795B and sc-106795C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

MAD2B siRNA (h) is recommended for the inhibition of MAD2B 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

MAD2B (F-12): sc-377367 is recommended as a control antibody for monitoring of MAD2B 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 MAD2B gene expression knockdown using RT-PCR Primer: MAD2B (h)-PR: sc-106795-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.