SANTA CRUZ BIOTECHNOLOGY, INC.

MAD2 siRNA (bovine): sc-270260

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, T47D, was shown to complete mitosis in the presence of nocodazole, an inhibitor of mitotic spindle assembly. MAD2 is localized to unattached kinetochores during prometaphase 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 IGFIR, 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 IGFIR. Evidence for release from the Insulin receptor after activation. J. Biol. Chem. 272: 10035-10040.
6. Liu, S.T., et al. 2003. Human CENP-I specifies localization of CENP-F, MAD1 and MAD2 to kinetochores and is essential for mitosis. Nat. Cell Biol. 5: 341-345.
7. Michel, L., et al. 2004. Complete loss of the tumor suppressor MAD2 causes premature cyclin B degradation and mitotic failure in human somatic cells. Proc. Natl. Acad. Sci. USA 101: 4459-4464.

CHROMOSOMAL LOCATION

Genetic locus: MAD2L1 (bovine) mapping to 6.

PRODUCT

MAD2 siRNA (bovine) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MAD2 shRNA Plasmid (bovine): sc-270260-SH and MAD2 shRNA (bovine) Lentiviral Particles: sc-270260-V as alternate gene silencing products.

For independent verification of MAD2 (bovine) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270260A, sc-270260B and sc-270260C.

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

MAD2 siRNA (bovine) is recommended for the inhibition of MAD2 expression in bovine 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

MAD2 (C-10): sc-374131 is recommended as a control antibody for monitoring of MAD2 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 MAD2 gene expression knockdown using RT-PCR Primer: MAD2 (bovine)-PR: sc-270260-PR (20 µl). 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.