

# MAD2 siRNA (h): sc-35837

## 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 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 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.

## CHROMOSOMAL LOCATION

Genetic locus: MAD2L1 (human) mapping to 4q27.

## PRODUCT

MAD2 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MAD2 shRNA Plasmid (h): sc-35837-SH and MAD2 shRNA (h) Lentiviral Particles: sc-35837-V as alternate gene silencing products.

For independent verification of MAD2 (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-35837A, sc-35837B and sc-35837C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

MAD2 siRNA (h) is recommended for the inhibition of MAD2 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  $\mu$ M in 66  $\mu$ 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.

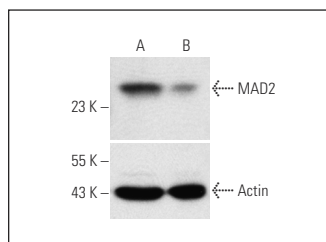
## GENE EXPRESSION MONITORING

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).

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MAD2 gene expression knockdown using RT-PCR Primer: MAD2 (h)-PR: sc-35837-PR (20  $\mu$ l, 451 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## DATA



MAD2 siRNA (h): sc-35837. Western blot analysis of MAD2 expression in non-transfected control (A) and MAD2 siRNA transfected (B) HeLa cells. Blot probed with MAD2 (C-19): sc-6329. Actin (I-19): sc-1616 used as specificity and loading control.

## SELECT PRODUCT CITATIONS

1. Huang, J.M., et al. 2010. Combination of vorinostat and flavopiridol is selectively cytotoxic to multidrug-resistant neuroblastoma cell lines with mutant TP53. *Mol. Cancer Ther.* 9: 3289-3301.
2. Dilshara, M.G., et al. 2019. Camptothecin induces mitotic arrest through MAD2-Cdc20 complex by activating the JNK-mediated Sp1 pathway. *Food Chem. Toxicol.* 127: 143-155.
3. Koban, F. and Freissmuth, M. 2023. The cell cycle protein MAD2 facilitates endocytosis of the serotonin transporter in the neuronal soma. *EMBO Rep.* 24: e53408.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.