

MDM2 siRNA (r): sc-63266

BACKGROUND

p53 is the most commonly mutated gene in human cancer identified to date. Expression of p53 leads to inhibition of cell growth by preventing progression of cells from G₁ to S phase of the cell cycle. Most importantly, p53 functions to cause arrest of cells in the G₁ phase of the cell cycle following any exposure of cells to DNA-damaging agents. The MDM2 (murine double minute-2) protein was initially identified as an oncogene in a murine transformation system. MDM2 functions to bind p53 and block p53-mediated transactivation of cotransfected reporter constructs. The MDM2 gene is amplified in a high percentage of human sarcomas that retain wildtype p53 and tumor cells that overexpress MDM2 can tolerate high levels of p53 expression. These findings argue that MDM2 overexpression represents at least one mechanism by which p53 function can be abrogated during tumorigenesis.

REFERENCES

1. Kastan, M.B., et al. 1991. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.* 51: 6304-6311.
2. Kastan, M.B., et al. 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD 45 is defective in ataxia-telangiectasia. *Cell* 71: 587-597.
3. Oliner, J.D., et al. 1993. Oncoprotein MDM2 conceals the activation domain of tumor suppressor p53. *Nature* 362: 857-860.
4. Haines, D.S., et al. 1994. Physical and functional interaction between wildtype p53 and MDM2 proteins. *Mol. Cell. Biol.* 14: 1171-1178.

CHROMOSOMAL LOCATION

Genetic locus: *Mdm2* (rat) mapping to 7q22.

PRODUCT

MDM2 siRNA (r) 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 MDM2 shRNA Plasmid (r): sc-63266-SH and MDM2 shRNA (r) Lentiviral Particles: sc-63266-V as alternate gene silencing products.

For independent verification of MDM2 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-63266A, sc-63266B and sc-63266C.

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

MDM2 siRNA (r) is recommended for the inhibition of MDM2 expression in rat 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

MDM2 (SMP14): sc-965 is recommended as a control antibody for monitoring of MDM2 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 MDM2 gene expression knockdown using RT-PCR Primer: MDM2 (r)-PR: sc-63266-PR (20 μ l, 426 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Busso, C.S., et al. 2009. Ubiquitination of mammalian AP endonuclease (APE1) regulated by the p53-MDM2 signaling pathway. *Oncogene* 28: 1616-1625.
2. Liu, Y., et al. 2012. SCYL1BP1 modulates neurite outgrowth and regeneration by regulating the MDM2/p53 pathway. *Mol. Biol. Cell* 23: 4506-4514.

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.