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p53 siRNA (m2): sc-44219

BACKGROUND

p53, a DNA-binding, oligomerization domain- and transcription activation domain-containing tumor suppressor, upregulates growth arrest and apoptosis-related genes in response to stress signals, thereby influencing programmed cell death, cell differentiation, and cell cycle control mechanisms. p53 localizes to the nucleus, yet can be chaperoned to the cytoplasm by the negative regulator, MDM2. MDM2 is an E3 ubiquitin ligase that is upregulated in the presence of active p53, where it poly-ubiquitinates p53 for proteasome targeting. p53 fluctuates between latent and active DNA-binding conformations and is differentially activated through posttranslational modifications, including phosphorylation and acetylation. Mutations in the DNA-binding domain (DBD) of p53, amino acids 110-286, can compromise energetically-favorable association with cis elements and are implicated in several human cancers.

REFERENCES

1. Banks, L., et al. 1986. Isolation of human p53-specific monoclonal antibodies and their use in the studies of human p53 expression. *Eur. J. Biochem.* 159: 529-534.
2. Hupp, T.R., et al. 1992. Regulation of the specific DNA-binding function of p53. *Cell* 71: 875-886.
3. Levine, A.J. 1997. p53, the cellular gatekeeper for growth and division. *Cell* 88: 323-331.
4. Ashcroft, M. and Vousden, K.H. 1999. Regulation of p53 stability. *Oncogene* 18: 7637-7643.

CHROMOSOMAL LOCATION

Genetic locus: Trp53 (mouse) mapping to 11 B3.

PRODUCT

p53 siRNA (m2) is a pool of 2 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 p53 shRNA Plasmid (m2): sc-44219-SH and p53 shRNA (m2) Lentiviral Particles: sc-44219-V as alternate gene silencing products.

For independent verification of p53 (m2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44219A and sc-44219B.

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

p53 siRNA (m2) is recommended for the inhibition of p53 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

p53 (A-1): sc-393031 is recommended as a control antibody for monitoring of p53 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 p53 gene expression knockdown using RT-PCR Primer: p53 (m2)-PR: sc-44219-PR (20 μ l, 521 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kleinridders, A., et al. 2009. PLRG1 is an essential regulator of cell proliferation and apoptosis during vertebrate development and tissue homeostasis. *Mol. Cell. Biol.* 29: 3173-3185.

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.