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p53 shRNA (m2) Lentiviral Particles: sc-44219-V

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, an E3 ubiquitin ligase that is upregulated in the presence of active p53, where MDM2 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, amino acids 110-286) of p53 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.
5. Soussi, T., et al. 2000. p53 website and analysis of p53 gene mutations in human cancer: forging a link between epidemiology and carcinogenesis. *Hum. Mutat.* 15: 105-113.
6. Chene, P. 2001. The role of tetramerization in p53 function. *Oncogene* 20: 2611-2617.
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CHROMOSOMAL LOCATION

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

PRODUCT

p53 shRNA (m2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see p53 siRNA (m2): sc-44219 and p53 shRNA Plasmid (m2): sc-44219-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

p53 shRNA (m2) Lentiviral Particles is recommended for the inhibition of p53 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

p53 (Pab 240): sc-99 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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