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EI24 shRNA (h) Lentiviral Particles: sc-43748-V

BACKGROUND

Inactivation of the tumor suppressor p53 occurs in more than 50% of human cancers. p53 functions in tumor suppression by monitoring DNA damage and executing pathways that negatively control cell growth. EI24 (also designated PIG8) is a protein produced by EI24, a DNA damage response gene involved in growth suppression and apoptosis. EI24 gene expression is specifically induced by wildtype p53, and overexpression suppresses cell growth by inducing apoptotic cell death. EI24 is highly conserved between mouse and human, and its expression in NIH/3T3 cells is induced following etoposide treatment. The EI24 gene is widely expressed in many human tissues including heart, liver, skeletal muscle and pancreas.

REFERENCES

- Greenblatt, M.S., et al. 1994. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res.* 54: 4855- 4878.
- Chen, X., et al. 1996. p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. *Genes Dev.* 10: 2438-2451.
- Lehar, S.M., et al. 1996. Identification and cloning of EI24, a gene induced by p53 in etoposide-treated cells. *Oncogene* 12: 1181-1187.
- Polyak, K., et al. 1997. A model for p53-induced apoptosis. *Nature* 389: 300-305.
- Levine, A. J. 1997. p53, the cellular gatekeeper for growth and division. *Cell* 88: 323-331.
- Gu, Z., et al. 2000. The p53-inducible gene EI24/PIG8 localizes to human chromosome 11q23 and the proximal region of mouse chromosome 9. *Cytogenet. Cell Genet.* 89: 230-233.

CHROMOSOMAL LOCATION

Genetic locus: EI24 (human) mapping to 11q24.2.

PRODUCT

EI24 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 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 EI24 siRNA (h): sc-43748 and EI24 shRNA Plasmid (h): sc-43748-SH as alternate gene silencing products.

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.

APPLICATIONS

EI24 shRNA (h) Lentiviral Particles is recommended for the inhibition of EI24 expression in human 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

EI24 (H-20): sc-11724 is recommended as a control antibody for monitoring of EI24 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EI24 gene expression knockdown using RT-PCR Primer: EI24 (h)-PR: sc-43748-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.