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### SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

[mail@szabo-scandic.com](mailto:mail@szabo-scandic.com)

[www.szabo-scandic.com](http://www.szabo-scandic.com)

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

# p73 shRNA (h2) Lentiviral Particles: sc-43730-V

## BACKGROUND

The p53 gene is a widely studied anti-oncogene, or tumor suppressor gene. The p53 gene product can act as a negative regulator of cell growth in response to DNA damage. Mutations and allelic loss of the p53 gene have been associated with malignant transformation in a wide variety of human tumors. p53 shares considerable sequence similarity with p73, a gene that maps to a region in chromosome 1 that is frequently deleted in neuroblastomas. However, p73 does not appear to be activated by DNA damaging agents. The p73 isoform  $\alpha$  inhibits drug-induced apoptosis in small cell lung carcinoma cells, while the p73 isoform  $\beta$  promotes it. p73 $\alpha$  also prevents Bax activation, mitochondrial dysfunction, caspase activation and is able to reduce apoptosis induced by the BH3-only protein, PUMA (p53 upregulated modulator of apoptosis). There is an equilibrium between p73 $\alpha$  and p73 $\beta$ , demonstrated by the fact that p73 $\alpha$  inhibits the pro-apoptotic effect of p73 $\beta$ .

## REFERENCES

1. Lane, D.P., et al. 1990. p53: oncogene or anti-oncogene? *Genes Dev.* 4: 1-8.
2. Malkin, D., et al. 1990. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 250: 1233-1238.
3. 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.
4. Jost, C.A., et al. 1997. p73 is a human p53-related protein that can induce apoptosis. *Nature* 389: 191-194.
5. Kaghad, M., et al. 1997. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell* 90: 809-819.
6. Schmale, H., et al. 1997. A novel protein with strong homology to the tumor suppressor p53. *Oncogene* 15: 1363-1367.
7. Reichelt, M., et al. 1999. The yeast two-hybrid system reveals no interaction between p73 $\alpha$  and SV 40 large T-antigen. *Arch. Virol.* 144: 621-626.

## CHROMOSOMAL LOCATION

Genetic locus: TP73 (human) mapping to 1p36.32.

## PRODUCT

p73 shRNA (h2) 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 p73 siRNA (h2): sc-43730 and p73 shRNA Plasmid (h2): sc-43730-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

p73 shRNA (h2) Lentiviral Particles is recommended for the inhibition of p73 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  lentiviral transducing particles per milliliter; contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

p73 (E-4): sc-17823 is recommended as a control antibody for monitoring of p73 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 p73 gene expression knockdown using RT-PCR Primer: p73 (h2)-PR: sc-43730-PR (20  $\mu$ l, 533 bp). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## 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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