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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) 

# IEX-1 shRNA (h) Lentiviral Particles: sc-43859-V

## BACKGROUND

Tumors are frequently observed as resistant to apoptotic induction by FAS, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) or irradiation. This anti-death activity may be attributed to immediate early-response genes that are regulated at the transcriptional level, including the protein IEX-1. IEX-1 (immediately early gene X-1), also known as IER3 (immediate early response 3), DIF-2 (differentiation-dependent gene 2 protein), immediate early protein GLY96 or PRG1 (PACAP-responsive gene 1 protein), is a 156 amino acid single-pass type II membrane protein that belongs to the IER3 family. IEX-1 was originally characterized as a gene induced by ultraviolet radiation and TNF- $\alpha$ , which protected human squamous carcinoma cells from apoptosis. Subsequent transfection studies have also shown that expression of IEX-1 in human keratinocytes and mouse fibroblasts results in more rapid proliferation of the cells as compared with controls. The promoter region of IEX-1 contains binding motifs for both NF $\kappa$ B and p53, suggesting that these proteins may regulate its expression.

## REFERENCES

1. Beyaert, R., et al. 1994. Molecular mechanisms of tumor necrosis factor-induced cytotoxicity. What we do understand and what we do not. *FEBS Lett.* 340: 9-16.
2. Kondratyev, A.D., et al. 1996. Identification and characterization of a radiation-inducible glycosylated human early-response gene. *Cancer Res.* 56: 1498-1502.
3. Wu, M.X., et al. 1998. IEX-1L, an apoptosis inhibitor involved in NF $\kappa$ B-mediated cell survival. *Science* 281: 998-1001.
4. Kumar, R., et al. 1998. A novel immediate early response gene, IEX-1, is induced by ultraviolet radiation in human keratinocytes. *Biochem. Biophys. Res. Commun.* 253: 336-341.

## CHROMOSOMAL LOCATION

Genetic locus: IER3 (human) mapping to 6p21.33.

## PRODUCT

IEX-1 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  $\mu$ 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 IEX-1 siRNA (h): sc-43859 and IEX-1 shRNA Plasmid (h): sc-43859-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

IEX-1 shRNA (h) Lentiviral Particles is recommended for the inhibition of IEX-1 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

IEX-1 (C-20): sc-8454 is recommended as a control antibody for monitoring of IEX-1 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 IEX-1 gene expression knockdown using RT-PCR Primer: IEX-1 (h)-PR: sc-43859-PR (20  $\mu$ l, 500 bp). 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](http://www.scbt.com) or our catalog for detailed protocols and support products.