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# fzr shRNA (h) Lentiviral Particles: sc-44349-V

## BACKGROUND

Fizzy-related protein, known as fzr, is a conserved eukaryotic gene that has been recently identified as a 7WD domain family member and is implicated in cell cycle regulation of *Drosophila* and yeast. Retroviral overexpression of fzr in B-lymphoma cells reduces tumor formation. fzr overexpression increases B-lymphoma cell susceptibility to natural killer cell (NK) cytotoxicity. fzr has been implicated in a new category of genes which suppress B-cell tumorigenesis. Current research suggests a novel role for fzr in the target cell interaction with NK cells. fzr also negatively regulates the levels of cyclins A, B and B3. Loss of fzr causes progression through an extra division cycle in the epidermis and inhibition of endoreduplication in the salivary gland, in addition to failure of cyclin removal. Conversely, premature fzr overexpression down-regulates mitotic cyclins, inhibits mitosis and transforms mitotic cycles into endoreduplication cycles.

## REFERENCES

1. Sigrist, S.J., et al. 1997. *Drosophila* fizzy-related downregulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. *Cell* 4: 671-681.
2. Inbal, N., et al. 1999. The mammalian Fizzy and Fizzy-related genes are regulated at the transcriptional and post-transcriptional levels. *FEBS Letts.* 3: 350-354.
3. Wang, C.X., et al. 2000. Overexpression of murine fizzy-related (fzr) increases natural killer cell-mediated cell death and suppresses tumor growth. *Blood* 1: 259-263.
4. Yudkovsky, Y., et al. 2000. Phosphorylation of Cdc20/fizzy negatively regulates the mammalian cyclosome/APC in the mitotic checkpoint. *Biochem. Biophys. Res. Commun.* 2: 299-304.
5. Zur, A., et al. 2001. Securin degradation is mediated by fzy and fzr, and is required for complete chromatid separation but not for cytokinesis. *EMBO J.* 4: 792-801.

## CHROMOSOMAL LOCATION

Genetic locus: FZR1 (human) mapping to 19p13.3.

## PRODUCT

fzr 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 fzr siRNA (h): sc-44349 and fzr shRNA Plasmid (h): sc-44349-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

fzr shRNA (h) Lentiviral Particles is recommended for the inhibition of fzr 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

fzr (B-6): sc-166714 is recommended as a control antibody for monitoring of fzr 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 fzr gene expression knockdown using RT-PCR Primer: fzr (h)-PR: sc-44349-PR (20  $\mu$ l, 542 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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