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# Nrf2 shRNA (h2) Lentiviral Particles: sc-44332-V

## BACKGROUND

The NF-E2 DNA binding protein is composed of two subunits, p45 and MafK. It regulates expression of globin genes in developing erythroid cells through interaction with Maf recognition elements (Mares). A family of NF-E2 related proteins, which are collectively known as the Cap "n" collar (CNC) family and include Nrf1 (also designated TCF11), Nrf2 and Nrf3, are bZIP transcription factors that heterodimerize with Maf proteins to bind Maf sequences. The Nrf proteins also bind the antioxidant response element (ARE) and are implicated in the regulation of detoxification enzymes and the oxidative stress response. They do so by heterodimerizing with Jun family members (c-Jun, Jun B and Jun D) to activate gene expression, specifically the detoxifying enzyme NQO1. The genes encoding Nrf1 and Nrf2 map to human chromosomes 17q21.32 and 2q31.2, respectively. Nrf2 is widely expressed and is thought to translocate to the nucleus after treatment with xenobiotics and antioxidants, which stimulate its release from its repressor protein, Keap1. The gene encoding human Nrf3 maps to chromosome 7p15.2. Nrf3 is highly expressed in placenta, B cells and monocytes.

## REFERENCES

1. Chan, J.Y., et al. 1995. Chromosomal localization of the human NF-E2 family of bZIP transcription factors by fluorescence *in situ* hybridization. *Hum. Genet.* 95: 265-269.
2. Chan, K., et al. 1996. Nrf2, a member of the NF-E2 family of transcription factors, is not essential for murine erythropoiesis, growth and development. *Proc. Natl. Acad. Sci. USA* 93: 13943-13948.
3. Kobayashi, A., et al. 1999. Molecular cloning and functional characterization of a new Cap 'n' collar family transcription factor Nrf3. *J. Biol. Chem.* 274: 6443-6452.
4. Chan, J.Y., et al. 2000. Impaired expression of glutathione synthetic enzyme genes in mice with targeted deletion of the Nrf2 basic-leucine zipper protein. *Biochim. Biophys. Acta* 1517: 19-26.

## CHROMOSOMAL LOCATION

Genetic locus: NFE2L2 (human) mapping to 2q31.2

## PRODUCT

Nrf2 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  $1.0 \times 10^6$  lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Nrf2 siRNA (h2): sc-44332 and Nrf2 shRNA Plasmid (h2): sc-44332-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

Nrf2 shRNA (h2) Lentiviral Particles is recommended for the inhibition of Nrf2 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

Nrf2 (A-10): sc-365949 is recommended as a control antibody for monitoring of Nrf2 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<sup>™</sup> compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker<sup>™</sup> 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<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Nrf2 gene expression knockdown using RT-PCR Primer: Nrf2 (h2)-PR: sc-44332-PR (20  $\mu$ l, 431 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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