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Keap1 shRNA (m) Lentiviral Particles: sc-43879-V

BACKGROUND

Keap1 (kelch-like ECH-associated protein 1, INrf2, KLHL19) is a stress sensing adaptor for the Cullin3 (Cul3)-dependent E3 ubiquitin ligase complex that negatively regulates Nrf2 (NF-E2-related factor 2). Steady state levels of proteins are under the influence of the ubiquitin pathway, which consists of ubiquitin activation (E1), conjugation (E2) and ligation (E3). Keap1 assembles into an E3 ubiquitin ligase complex with Cul3 and Rbx1 and targets lysine residues in the N-terminal Neh2 domain of Nrf2 for ubiquitin conjugation. The Keap1-Nrf2 system mediates cytoprotective gene expression in response to oxidative and/or electrophilic stresses. Keap1 constitutively suppresses Nrf2 activity under unstressed conditions, oxidants or electrophiles provoke the repression of Keap1 activity, inducing Nrf2 activation. Cys 273 and Cys 288 residues of Keap1 are required for suppressing Nrf2 nuclear accumulation. Keap1 sequesters Nrf2 in the cytoplasm through an active Crm1/exportin-dependent nuclear export mechanism.

REFERENCES

- Zhang, D.D., et al. 2003. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. *Mol. Cell. Biol.* 23: 8137-8151.
- Kobayashi, A., et al. 2004. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol. Cell. Biol.* 24: 7130-7139.
- Zhang, D.D., et al. 2004. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol. Cell. Biol.* 24: 10941-10953.
- Devling, T.W., et al. 2005. Utility of siRNA against Keap1 as a strategy to stimulate a cancer chemopreventive phenotype. *Proc. Natl. Acad. Sci. USA* 102: 7280A-7285A.

CHROMOSOMAL LOCATION

Genetic locus: Keap1 (mouse) mapping to 9 A3.

PRODUCT

Keap1 shRNA (m) 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 Keap1 siRNA (m): sc-43879 and Keap1 shRNA Plasmid (m): sc-43879-SH as alternate gene silencing products.

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.

APPLICATIONS

Keap1 shRNA (m) Lentiviral Particles is recommended for the inhibition of Keap1 expression in mouse 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

Keap1 (E-20): sc-15246 is recommended as a control antibody for monitoring of Keap1 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 Keap1 gene expression knockdown using RT-PCR Primer: Keap1 (m)-PR: sc-43879-PR (20 μ l, 509 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.