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SEN8 shRNA (h) Lentiviral Particles: sc-44452-V

BACKGROUND

SUMO (small ubiquitin-related modifier), a member of the ubiquitin-like protein family, regulates diverse cellular functions of a variety of target proteins, including transcription, DNA repair, nucleocytoplasmic trafficking and chromosome segregation. SUMO precursor proteins undergo cleavage of the residues after the "GG" region by SUMO-specific proteases in maturation. This cleavage of the precursor is a prerequisite for subsequent sumoylation. The sentrin-specific (or SUMO-specific) protease (SEN) proteins belong to the peptidase C48 family and include SENP1-3 and SENP5-8. SENP1, SENP2 and SENP3 degrade UBL1 and SMT3H2 conjugates and subsequently release the monomers from sumoylated substrates. HIPK2 is a desumoylation target for SENP1 which shuttles between the cytoplasm and the nucleus. Mutation analyses reveal that SENP1 contains the nuclear export sequence (NES) within the extreme carboxyl-terminal region, and SENP1 is exported to the cytoplasm in a NES-dependent manner. SENP2 has been implicated as a downregulator of CTNNB1 levels and may therefore be a modulator of the Wnt pathway. SUMO protease SENP3 reverses the sumoylation of MEF2 to augment its transcriptional and myogenic activities. SENP5 localizes to the nucleolus and preferentially processes SUMO-3. It is thought to play a role in mitosis and/or cytokinesis. SENP6 localizes to the cytoplasm and releases SUMO-1. Expression of SENP6 is higher in reproductive organs, indicating that it may mediate processes related to reproduction. SENP8 is involved in the release of sentrins.

REFERENCES

- Gong, L., et al. 2000. Differential regulation of sentrinized proteins by a novel sentrin-specific protease. *J. Biol. Chem.* 275: 3355-3359.
- Kim, K.I., et al. 2000. A new SUMO-1-specific protease, SUSP1, that is highly expressed in reproductive organs. *J. Biol. Chem.* 275: 14102-14106.
- Cheng, J., et al. 2004. SENP1 enhances androgen receptor-dependent transcription through desumoylation of histone deacetylase 1. *Mol. Cell. Biol.* 24: 6021-6028.
- Reverter, D., et al. 2004. A basis for SUMO protease specificity provided by analysis of human SENP2 and a SENP2-SUMO complex. *Structure* 12: 1519-1531.

CHROMOSOMAL LOCATION

Genetic locus: SENP8 (human) mapping to 15q23.

PRODUCT

SEN8 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 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 SENP8 siRNA (h): sc-44452 and SENP8 shRNA Plasmid (h): sc-44452-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.

APPLICATIONS

SEN8 shRNA (h) Lentiviral Particles is recommended for the inhibition of SENP8 expression in human 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

SEN8 (E-11): sc-271498 is recommended as a control antibody for monitoring of SENP8 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 SENP8 gene expression knockdown using RT-PCR Primer: SENP8 (h)-PR: sc-44452-PR (20 μ l). 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

See our web site at www.scbt.com or our catalog for detailed protocols and support products.