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TDG shRNA (h) Lentiviral Particles: sc-44142-V

BACKGROUND

In the DNA of higher eukaryotes, hydrolytic deamination of 5-methylcytosine to thymine leads to the formation of G/T mismatches. G/T mismatch-specific Thymine DNA Glycosylase (TDG) is a nuclear protein which corrects G/T mismatches to G/C pairs by hydrolyzing the carbon-nitrogen bond between the sugar-phosphate backbone of the DNA and the mispaired thymine. TDG also corrects a subset of G/U mispairs inefficiently removed by the more abundant uracil glycosylases. Retinoic acid receptors interact physically and functionally with TDG, enhancing the ability of the retinoid X receptor and the retinoid X receptor/retinoic acid receptor complex to bind to their response elements. TDG interacts with, and is covalently modified by, the ubiquitin-like proteins SUMO-1 and SUMO-2/3, resulting in a reduction of the DNA substrate and AP site binding affinity of TDG. This sumoylation is associated with a significant increase in enzymatic turnover in reactions with a G/U substrate and the loss of G/T processing activity.

REFERENCES

1. Neddermann, P., et al. 1994. Efficient removal of uracil from G/U mispairs by the mismatch-specific thymine DNA glycosylase from HeLa cells. *Proc. Natl. Acad. Sci. USA* 91: 1642-1646.
2. Um, S., et al. 1998. Retinoic acid receptors interact physically and functionally with the G/T mismatch-specific thymine-DNA glycosylase. *J. Biol. Chem.* 273: 20728-20736.
3. Privezentzev, C.V., et al. 2001. The HAP1 protein stimulates the turnover of human mismatch-specific thymine-DNA-glycosylase to process 3,N(4)-ethenocytosine residues. *Mutat. Res.* 480-481: 277-284.
4. Hardeland, U., et al. 2002. Modification of the human thymine-DNA glycosylase by ubiquitin-like proteins facilitates enzymatic turnover. *EMBO J.* 21: 1456-1464.
5. SWISS-PROT/TrEMBL (Q13569). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: TDG (human) mapping to 12q23.3.

PRODUCT

TDG 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 TDG siRNA (h): sc-44142 and TDG shRNA Plasmid (h): sc-44142-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

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

TDG (D-11): sc-376652 is recommended as a control antibody for monitoring of TDG 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 TDG gene expression knockdown using RT-PCR Primer: TDG (h)-PR: sc-44142-PR (20 μ l, 536 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.

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