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SANTA CRUZ BIOTECHNOLOGY, INC.

TDG siRNA (h): sc-44142

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/retinoid 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. and Jiricny, J. 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., Harbers, M., Benecke, A., Pierrat, B., Losson, R. and Chambon, P. 1998. Retinoic acid receptors interact physically and functionally with the G/T mismatch-specific thymine-DNA glycoslyase. J. Biol. Chem. 273: 20728-20736.
3. Privezentzev, C.V., Saparbaev, M., and Laval, J. 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., Steinacher, R., Jiricny, J., and Schar, P. 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 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TDG shRNA Plasmid (h): sc-44142-SH and TDG shRNA (h) Lentiviral Particles: sc-44142-V as alternate gene silencing products.

For independent verification of TDG (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-44142A, sc-44142B and sc-44142C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TDG siRNA (h) is recommended for the inhibition of TDG expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

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 µl, 536 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Métivier, R., et al. 2008. Cyclical DNA methylation of a transcriptionally active promoter. Nature 452: 45-50.