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# 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/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. 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 glycosylase. *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  $\mu$ M 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M 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  $\mu$ M in 66  $\mu$ l. 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  $\mu$ l, 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.